TO PUP OR NOT TO PUP? USING PHYSIOLOGY AND DIVE BEHAVIOR TO ANSWER THE WEDDELL SEAL'S OVERWINTER QUESTION

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A

THESIS

Presented to the Faculty

of the University of Alaska Fairbanks

in Partial Fulfillment of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

By

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Fairbanks, Alaska

August 2015

Abstract

Female Weddell seals (*Leptonychotes weddellii*) haul-out on the fast-ice surrounding the Antarctic continent in October and November each year to give birth to and nurse their pups. Breeding follows directly after weaning (December) and the annual molt begins in January-February. Animals reduce foraging efforts during the lactation and molting periods, but very little is known regarding the influence of this reduced activity on physiological condition. After a period of embryonic diapause, the annual molt coincides with embryo attachment and the start of active gestation. Consequently, female physiological condition at this time may influence reproductive success the following year. Overall female health and the ability to forage successfully throughout the gestation period (austral winter) may impact the likelihood that a pregnancy is brought to term. Therefore, this study tested whether overwinter changes in Weddell seal physiology and foraging efforts are reflected in reproductive outcomes the following year (i.e., to answer the over winter question of "to pup or not to pup?").

From 2010-2012, 100 (January-February: $n = 53$; October-November: $n = 47$) adult female Weddell seals were captured in Erebus Bay, Antarctica to assess overwinter changes in physiological condition and/or dive behavior that may be associated with reproductive success. Morphometric measurements and isotopic dilution procedures revealed that female Weddell seals gain ~10-15% of their body mass across the winter period, primarily in the form of blubber and lipid mass. The proportion of mass and lipid gain was similar regardless of whether females returned the following year and successfully gave birth, or did not produce a pup. Further, the amount of mass and energy acquired across gestation in the Weddell seal was markedly less than previously reported for other phocid species. Despite changes in activity patterns and body composition, Weddell seals maintained blood hemoglobin and muscle myoglobin concentrations across the winter. Therefore, Weddell seal total body oxygen stores and calculated aerobic dive limit (cADL) were conserved. This ensures that females have the physiological capabilities to effectively forage directly following the annual molt when they are at their leanest and must regain body mass and lipid stores. Although aerobic capacities did not change, dive effort varied considerably throughout the austral winter. Proxies of dive effort (duration, depth, %dives > cADL) were highest just after the molt (January-February) and just prior to the subsequent pupping season (August-September). Additionally, the proportion of each day spent diving

increased mid-winter. Females that were observed the following year with a pup significantly increased all indices of foraging effort during the austral winter as compared to females that returned without a pup. This study is the first to identify and measure differences in dive efforts due to reproductive status, and indicates that successful reproduction is associated with greater foraging effort.

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Acknowledgements

I would like to thank my advisor, Dr. Jennifer Burns, for all her time and support through this entire process as a graduate student. I came to Anchorage for a one summer internship after my sophomore year of college, and somehow – I'm still not entirely sure how – the experience turned into multiple summers, and a doctoral degree program. Six years later (2 undergraduate, 4 graduate), I can easily say that joining Jenn's lab has led to adventures better than anything I ever could have imagined, and one of the best decisions I've ever made. I am very grateful for all the opportunities and doors that Jenn has opened for me. Through all the field seasons, coffee breaks, office visits, and airports all across the world, her time, guidance, friendship, and high expectations have made me a much better scientist and person.

I'd also like to thank the other members of my committee, Drs. Jo-Ann Mellish, Daniel Costa, Loren Buck, and Sarah Hardy for all their support – providing constructive comments to the data chapters in this dissertation, additional field experiences, and graciously (…or perhaps unknowingly) providing all the almond Snickers bars to fuel the making of this dissertation (Dan).

I am extremely grateful to my Ice friends and family, all of whom this work could not have been done without their help. In particular, I wanted to thank my "Team Type A" partners in crime – Kim Goetz and Linnea Pearson for all their intense organizational skills and late nights processing samples with me, and Luis Hückstädt, Patrick Robinson, Michelle LaRue, and Brad Buckley for all their help in the field. Additionally, the many UAA/UAF graduate students have made living in Anchorage so enjoyable and provided lots of help troubleshooting assays, especially Melanie Richter, Danielle Dillon, Veronica Padula, and my labmates Roxanne Beltran and Amy Kirkham.

We have the most amazing support doing our work at McMurdo Station, and none of this research would have been possible without Raytheon Polar Services and Lockheed Martin ASC support staff in Christchurch, NZ and McMurdo Station.

I am thankful for my very supportive family. Despite Alaska and Antarctica being a long ways from home, they've offered their help at every turn.

This research was supported by NSF ANT-0838937 to J. Burns and ANT-0838892 to D. Costa. This material is based upon work supported by the National Science Foundation Graduate Research Fellowship under Grant No. DGE-1242789. Any opinion, findings and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation. Additional support was provided through a UAF Center for Global Change Student Research Grant with funds from the Cooperative Institute for Alaska Research and Alaska Climate Science Center, the Environment and Natural Resources Institute (ENRI), Alaska LGL, and travel funds from Alaska IDeA Network of Biomedical Research Excellence (INBRE). All work was conducted under Marine Mammal permit No. 87-1851-04 issued by the Office of Protected Resources, National Marine Fisheries Service, UAA and UCSC IACUC approvals, and Antarctic Conservation Act permits.

Preface

Most of the chapters in this dissertation have been published or submitted to peer review journals. All were submitted with co-authors that contributed significantly to this collaborative work. However, all chapters presented in this dissertation are my own work as primary author.

Chapter 2 was first presented at the 2013 Society for Integrative & Comparative Biology Meeting in San Francisco and was published in 2014 in PLoS ONE. The project idea was first generated after realizing that current morphometric techniques significantly overestimated measured Weddell seal body mass – after much delibration as to the underlying causes of this discrepancy, we finally came up with a solution. This paper was co-authored with Linnea Pearson, Daniel Costa, and Jennifer Burns who all helped to collect these data.

Chapter 3 has been presented at numerous national and international conferences (American Physiological Society in Colorado in 2010, Society for Integrative & Comparative Biology in 2013, the Biennial Marine Mammal Conference in Tampa 2011, the Society for Experimental Biology in Valencia 2013, the Scientific Committee on Antarctic Research in Barcelona 2013, and American Physiological Society in San Diego in 2014) and was published in 2015 in Functional Ecology. This manuscript was co-authored by Riley Krotz and Julie Avery for their involvement with hormone analyses and Daniel Costa and Jennifer Burns.

Chapter 4 was presented in combination with other chapters at scientific meetings and has been accepted for publication at the Journal of Comparative Physiology B with Daniel Costa and Jennifer Burns.

Chapter 5 was first presented at the 5th International Bio-logging Science Symposium in Strasbourg, France, 2014 and was also be presented at the Annual Animal Behavior Society conference as part of the Marine Mammal symposium in Anchorage during the summer of 2015. This manuscript has been prepared for submission to Marine Ecology Progress Series with co-authors Kimberly Goetz, Daniel Costa, and Jennifer Burns. The dive data from satellite-relay loggers used for this work have been part of a much larger, collaborative study assessing overwinter habitat utilization in Weddell seals, and this work was conducted working closely with our collaborators at U.C. Santa Cruz.

Chapter 1. General Introduction

1.1 Antarctic Ecology & Weddell Seal Life History Patterns

Polar marine mammals endure some of the most rapidly-changing environments in the world with large-scale seasonal changes in productivity [1]. The survival and reproductive success of these species rely on the individual's ability to "make a living" despite large-scale intra- and inter-annual variation in habitat and resources. In addition to drastic seasonal patterns in primary productivity, El Niño Southern Oscillation (ENSO), the Antarctic Circumpolar Wave, and large iceberg calving events (most notably B-15 [2]), can temporarily alter ocean currents and ice extent. Both predictable (seasonal) and unpredictable (episodic) changes in environmental conditions can induce shifts in prey availability, and are apparent in the foraging behavior, reproductive success, and survivorship of higher trophic levels [3–5]. Pinnipeds often travel large distances in order to reach particularly productive water masses, or hotspots where prey resources are concentrated, such as upwelling that occurs at shelf breaks, seamounts, eddies, and circumpolar deep water [6–9]. These animals have life history patterns that reflect the strong seasonal changes in the environment and often exhibit extreme fluctuations in behavior and physiology [10,11].

Weddell seals (*Leptonychotes weddellii*) are the southernmost living mammal in the world, and are also top predators with a circumpolar distribution around the Antarctic continent. The Weddell seal population in close proximity to McMurdo Station (Erebus Bay, Ross Sea) is comprised of ~2,000 individuals at the southernmost extent of the species' range [12]. Fast ice surrounds the continent for 8-9 months of the year (20×10^6 km^2) and declines rapidly during the austral summer (4×10^6 km^2; [13,14]). The extent as well as timing of sea ice melting and cross-shelf nutrient exchange affect the annual cycles of nutrient availability for summer biological productivity [15]. These annual cycles in productivity and the Southern Oscillation Index (SOI) are strongly correlated with Antarctic pinniped reproductive rates, population estimates, and cohort strength [3]. In particular, Weddell seal reproductive rates have been remarkably in phase with the SOI since the 1970's, with 4-5 year fluctuations in reproductive rates [3]. Weddell seals across the Antarctic region, in McMurdo Sound, Macquarie Island, and the Antarctic Peninsula

show simultaneous fluctuations in populations, further supporting that large-scale oceanographic systems are likely responsible for the cyclical changes in pinniped numbers.

During most of the year, Weddell seals spend >50% of each day diving, but during the austral spring, females start to haul-out on the fast-ice around the Antarctic continent to give birth to their pups. Lactation lasts for approximately 6-7 weeks, during which time females markedly reduce foraging efforts [16]. Weaning is immediately followed by the breeding season (December; [17]), and annual molt (January-February; [18]). Weddell seals also spend much more time hauled-out on the ice during the molting period, presumably because the process of epidermal and hair regrowth proceeds more quickly when skin temperatures stay elevated [19,20]. Therefore, Weddell seal foraging activities and energy budgets change drastically over the summer breeding and molting seasons, with seals expending large amounts of energy for lactation and hair regeneration, but reducing diving and foraging activities. Post-molt, Weddell seals increase foraging efforts during the eight-month overwinter period, with some animals remaining in close vicinity to their breeding colonies [21] while others travel >1,000 km to more productive water masses [22]. The observed changes in Weddell seal population numbers in Erebus Bay are not well correlated with ice conditions in the spring, but instead appear to be dependent on prey availability or predictability of fish aggregates throughout the austral winter [3]. Despite evidence that the overwinter foraging period would be a critical time for female Weddell seals to regain condition for successful reproduction and maintenance of population numbers, most of the research on this species has been conducted after periods of extended foraging activity, in the austral spring (October/November) during the lactation period [23–26]. In contrast, much less research has focused on the period following the annual molt when animals would likely be in relatively poor condition (due to loss of lipid stores), and at the start of the long winter foraging effort.

1.2 Weddell Seal Physiology

1.2.1 Body Composition

Traditionally, Weddell seals, like other phocids, were thought to utilize a capital breeding strategy [27]. Under this scenario, females fast throughout the lactation period, relying solely on energy stores acquired during gestation to support self-maintenance costs and neonatal growth [27]. This is in contrast to income-breeding pinnipeds that alternate nursing their pups with foraging for days to weeks throughout the lactation period. Female capital-breeding phocids give birth on beaches or ice, away from their marine food resources, so the female's ability to remain with her pup and offer it provisions directly correlates with the size of her own energy reserves [28,29]. Furthermore, larger females have lower mass-specific metabolic rates than smaller females, giving larger seals an advantage in that less of their energy reserves must be allocated towards self-maintenance and more energy can be devoted to the pup [28]. More recently, Weddell seals have been observed to resume foraging mid-lactation (~25% of the day diving; [16]), and efforts are higher in smaller and leaner females [30]. Despite utilizing a mixed income-capital breeding strategy, Weddell seals still lose ~30% of their body mass across lactation, similar to other phocid species [31–35]. Mass lost is primarily in the form of lipid mass; however, catabolism of some protein stores is required in order to meet metabolic demands for carbohydrate and protein fuels, as well as for the production of new hair during the molt [31].

The molt period is known to be associated with marked mass and lipid loss in other phocids [36,37], but changes in body composition during this period have yet to be measured in Weddell seals. Because the molt coincides with embryo implantation and the start of active gestation for the next year's reproductive effort [38–40], body size and condition during the molt may influence the probability of embryo implantation and maintenance of early pregnancy [41,42]. Following the molt, the overwinter foraging period is likely important to the recuperation of body mass and lipid stores necessary for the next year's reproductive events. Animals that are unable to regain enough energy across the overwinter foraging period to support gestation and/or lactation due to environmental perturbations or physiological constraints may have lower reproductive success.

Numerous hormones regulate energy allocation during times of re-feeding and also fasting, and many studies have assessed hormonal correlates with body composition and energy deposition [43–49]. Typically, hormone profiles indicative of suppressed metabolic rates (decreased thyroid hormone concentrations), protein sparing (high growth hormone; low insulin-like growth factor-1), and increased stress levels (cortisol) accompany fasting [50–54]. Much less work has been conducted during times of re-feeding and gestation to assess how hormone profiles are responsible for allocating energy acquired during foraging for self-maintenance or building reserves [54–57]. These underlying mechanisms would likely play a role in tissue accretion during the post-molt foraging period for adequate overwinter recuperation. Hormones associated with energy allocation have also been shown to strongly influence the process of embryo attachment and to support fetal growth [58], and may influence the likelihood of producing a pup the subsequent year. Moreover, the combination of body mass, composition, and hormone changes have the ability to profoundly impact the diving ability of Weddell seals, once active foraging resumes following the annual molt [59–62].

1.2.2 Oxygen Stores & the Aerobic Dive Limit

Weddell seals, like all diving mammals, rely heavily on the oxygen (O_2) stores that they carry to depth in order to forage effectively [63–66]. Therefore, marine mammals have much larger O_2 stores than terrestrial mammals of similar size, and these are highly correlated with dive durations [66–68]. For example, the deep-diving phocid seals have hemoglobin concentrations (Hb) that are 2x those of terrestrial mammals, 1.5x hematocrit values, 3x larger blood volumes, and 10-20x larger muscle myoglobin loads (Mb) [69–71] as compared to terrestrial mammals. Large O_2 stores in combination with low diving metabolic rates maximize the aerobic dive limit (ADL), the time that can be spent underwater while utilizing aerobic respiration [72,73].

Marine mammals have a suite of physiological adaptations, the graded "dive response", that allow diving metabolic rates to only be slightly elevated above resting during long dives. At the onset of a long dive, animals become bradycardic and can decrease heart rates to < 10 bpm [74]. At the same time, marine mammals employ peripheral vasoconstriction, shunting blood away from the muscles. This prevents Hb-O_2 dissociation and transfer of blood-O_2 to muscle Mb. Another important aspect of the dive response is the parsimonious use of muscle and blood O_2,

since depleting one store long before the other would shorten dives [75]. Further, pinnipeds cannot exhaust blood O_2 stores completely, as even in the longest dives that far exceed the ADL animals require O_2 for the heart, lungs, and brain [76,77]. Despite reduced muscular perfusion, animals are able to exercise and forage because muscles predominantly rely on their oxy-Mb stores for aerobic metabolism. This reliance on oxidative metabolism is reflected by high mitochondrial densities, high lipid stores for fuels, predominantly oxidative fiber types, and slow flux of metabolites through the TCA cycle (conserving fuel) in marine mammal muscles [70,78]. To decrease diving metabolic rates and conserve O_2 stores, animals can also reduce body temperatures and employ stroke and glide mechanisms, or simply allow themselves to passively drift through the water column [79].

Production of red blood cells and O_2 carrying proteins such as blood Hb and muscle Mb that are essential components of the diving ability of marine mammals is regulated in part by exercise and hypoxia exposure, and levels can decrease with disuse [80–83]. Similarly, muscle structural and biochemical properties associated with endurance activities atrophy during times of inactivity in many terrestrial mammals [84–86]. If Weddell seals were to experience the same wasting of muscle structure and O_2 storage proteins as is typically seen in other mammals after prolonged inactivity, this would result in reduced foraging capabilities during the initiation of winter foraging. Because this foraging period is likely critical for regaining body mass and condition (lipid stores) lost during the molt, in preparation for the next year's reproductive effort [28,34,87], any changes in prey resources or ice conditions during late summer/fall would likely have the largest impact on Weddell seals. Impacts would be particularly profound if physiological condition were poor and/or aerobic capacities compromised at the end of the austral summer. However, hibernating mammals that must forage and escape predation immediately after emergence, are largely resistant to the negative physiological effects of inactivity and fasting that are common in other mammals [88,89], and some marine mammal species may have a similar physiological resistance to reduced activity and maintain aerobic capacities [62,90].

1.3 Links Between Physiology and Dive Behavior

The advances in biotelemetric techniques have provided valuable tools to identify the correlations between physiology and foraging success, given previously developed proxies of efficient, or "optimal", diving practices. Pinnipeds are considered central place foragers, as all seals must return to the surface for air after a dive [91]. Animals should maximize time spent at a rich prey patch, but optimal dive times also reflect the fact that longer dives require more recuperation time at the water's surface. The marginal value theorem (MVT) balances this need to acquire patchy prey resources against depleting O_2 stores during longer dives [91–93]. According to the MVT, animals should spend time foraging within a patch until the overall energetic gain falls to average values for that area. Animals that spend longer travelling to depth to reach a prey patch should spend more time there. However, because animals only have limited O_2 stores and exceeding the ADL leads to build-up of anaerobic byproducts, there is a cap on how much time can be spent in a patch as animals must return to the surface for air.

Because dives that fall within the ADL are much more efficient than dives that exceed the ADL, changes in ADL times across the year would likely be reflected by different diving behaviors. Animals that dive longer may be able to exploit different prey resources at greater depths; however, this tactic comes at a cost. Anaerobic metabolism only produces 1/18th the amount of ATP as aerobic metabolism and is unsustainable for long periods. Once a seal surpasses its ADL and local O_2 stores are depleted, blood lactate levels increase upon resurfacing. This requires longer post-dive surface time to re-establish internal homeostasis [72,73]. Therefore, spending more time at the water's surface after long dives decreases the total amount of time that can ultimately be spent diving. Numerous isolated hole experiments revealed that free-ranging Weddell seals typically exceed their ADL on just ~10% of dives [72,73]. Further, isolated hole studies showed that the ADL can be accurately calculated by dividing endogenous O_2 stores in the tissues by diving metabolic rate in cases where taking blood samples directly following dives cannot be used to measure the "true" ADL (i.e., diving lactate threshold) [73]. Still, we do not know how the variation in calculated ADLs and other physiological parameters (i.e., lipid stores, total mass, muscle proteins) among animals, actually correlate with diving behaviors on an individual basis.

Body mass is a major determinant of diving capabilities in marine mammals, as O_2 stores scale isometrically, and diving metabolic rate scales allometrically ($Mass^{0.75}$) with body mass [94–96]. Therefore, for given O_2 stores, larger animals should have longer cADLs and be able to make longer dives [62,97]. This pattern is seen in pinniped species that exhibit sexual size-dimorphism, and the larger sex makes longer and deeper dives [98,99]. However, the foraging depths targeted and therefore the average dive duration relative to the cADL, has the potential to change across the year. Especially in polar regions, light availability and primary productivity decrease substantially during the winter months. Consequently, prey capture success rates may decline during periods of low-light conditions [100], and marine mammals may need to increase foraging efforts to obtain equivalent amounts of prey. Further, mammal species increase food intake by ~10-15% during gestation [101], suggesting that gestating Weddell seals will also need to increase foraging efforts across the austral winter relative to their non-reproductive counterparts to maintain body condition and sustain the pregnancy [41,42].

1.4 Scope of This Study

The primary objectives of this dissertation are to assess seasonal plasticity in female Weddell seal (1) physiological condition and (2) diving behavior indicative of foraging effort, with the ultimate aim of linking these parameters with subsequent reproductive success. To achieve these goals, I measure body composition (lipid), related hormone profiles, total body oxygen stores, and muscle biochemistry in post-molt Weddell seals. These values are then compared to the physiological status of pre-breeding females following the austral winter during years 2010-2012. Further, dive behavior was monitored across the austral winter, to assess whether there are key periods in the winter that may be important for recuperation of body mass and subsequent reproductive success. Finally, I aim to determine whether animals that returned the following year and successfully produced a pup differ in measureable ways from females that did not give birth.

Chapter 2, "Improving the precision of our ecosystem calipers: A modified morphometric technique for estimating marine mammal mass and body composition" is a methods paper that examines the pros and cons of using different techniques to determine marine mammal body

composition. I compare measures of body composition derived from morphometric truncated cones calculations to those from isotopic dilution after preliminary work showed a large discrepancy between these two commonly used methods. This work shows that current morphometric techniques overestimate measured Weddell seal body mass by >25%, and in this chapter, I develop a new method that utilizes elliptical cones to more accurately estimate animal total body and blubber mass. This chapter also provides model coefficients to relate morphometric and isotopic dilution techniques, and identify key measurements that could be used as proxies of animal size and condition, if handling times need to be kept short. This paper has been published in PLoS ONE (2014; 9: e91233).

In chapter 3, "How do overwinter changes in body composition and hormone profiles influence Weddell seal reproductive success?", changes in total body and lipid mass are measured across the austral winter, in combination with hormone profiles responsible for energy allocation and accretion of new tissues. These measures test the hypothesis that gestating female Weddell seals gain more mass and lipid stores overwinter to prepare for lactation, as compared with females that returned to Erebus Bay the following the year without a pup. This study also assesses whether Weddell seals gain less mass and condition across the post-molt foraging period (and gestation) relative to other phocid species. Finally, this chapter examines whether hormones that influence energy allocation (i.e., thyroid hormones, cortisol) also promote positive reproductive outcomes. Understanding physiological factors that influence the probability of reproduction is important for assessing the species' vulnerability to changes in ecosystem structure. This chapter has been published and is available at Functional Ecology (2015; doi: 10.1111/1365-2435.12434).

Animal size determines how much O_2 can be carried to depth, as well as the rate at which these stores are used. Therefore, chapter 4 assesses whether the aerobic capacity of Weddell seals atrophies by the end of the annual molt when animals are in relatively poor condition. To test if disuse induces muscular atrophy, I also examine changes in muscle structure (myosin heavy chain composition) and catabolic enzyme activities (citrate synthase, β-hydroxyacyl-CoA dehydrogenase, lactate dehydrogenase) in females at the end of the annual molt and after the overwinter diving period. Such comparisons of total body O_2 stores among individuals typically rely on using total body mass as a scalar. However, it has already been made clear that Weddell

seals exhibit seasonal changes in body composition (i.e., lipid and blubber compartments that do not store O_2). Therefore, I assess whether adding body composition and only scaling O_2 values to lean body mass significantly adds to statistical models when assessing seasonal O_2-store changes. "Scaling matters: Incorporating body composition into Weddell seal seasonal oxygen store comparisons reveals maintenance of aerobic capacities" has been accepted for publication in the Journal of Comparative Physiology B (doi: 10.1007/s00360-015-0922-8).

The final thrust of this project is to examine how Weddell seal physiological condition correlates with diving behavior throughout the austral winter, and these links are made in chapter 5. Body size and composition, O_2 stores, and muscular efficiency all affect how long Weddell seals are able to remain underwater, foraging. In this chapter, I assess whether animals increase foraging efforts (i.e., dive duration, depth, bottom time, longer dive bouts) and/or surpass their cADL more often during certain times of the year, which would indicate that these periods are especially critical to recuperating body mass, or that prey abundance or quality is particularly low. Because the costs of maintaining a fetus are the only differences in energetic requirements between gestating and non-pregnant Weddell seals across the post-molt foraging period, I test whether dive efforts are higher in pregnant females throughout the austral winter. This chapter, "Temporal changes in Weddell seal dive behavior over winter: Are females changing foraging tactics to support gestation?" links all the physiological parameters reported in previous chapters to dive performance, body mass recuperation, and reproductive success. This paper has been prepared for submission to the Marine Ecology Progress Series.

The concluding chapter in this dissertation, "Physiological condition and dive behavior across the gestation period in Weddell seals: Links with reproductive success of a high-latitude predator; Conclusions" integrates the key findings from the entire thesis, and highlights the significance of this dissertation. Chapter 6 synthesizes the impacts that changes in physiology have on Weddell seal foraging patterns and reproduction. Finally, I highlight opportunities for future research that would provide valuable insight into factors that are important to acquiring resources from a rapidly changing environment and to reproductive success.

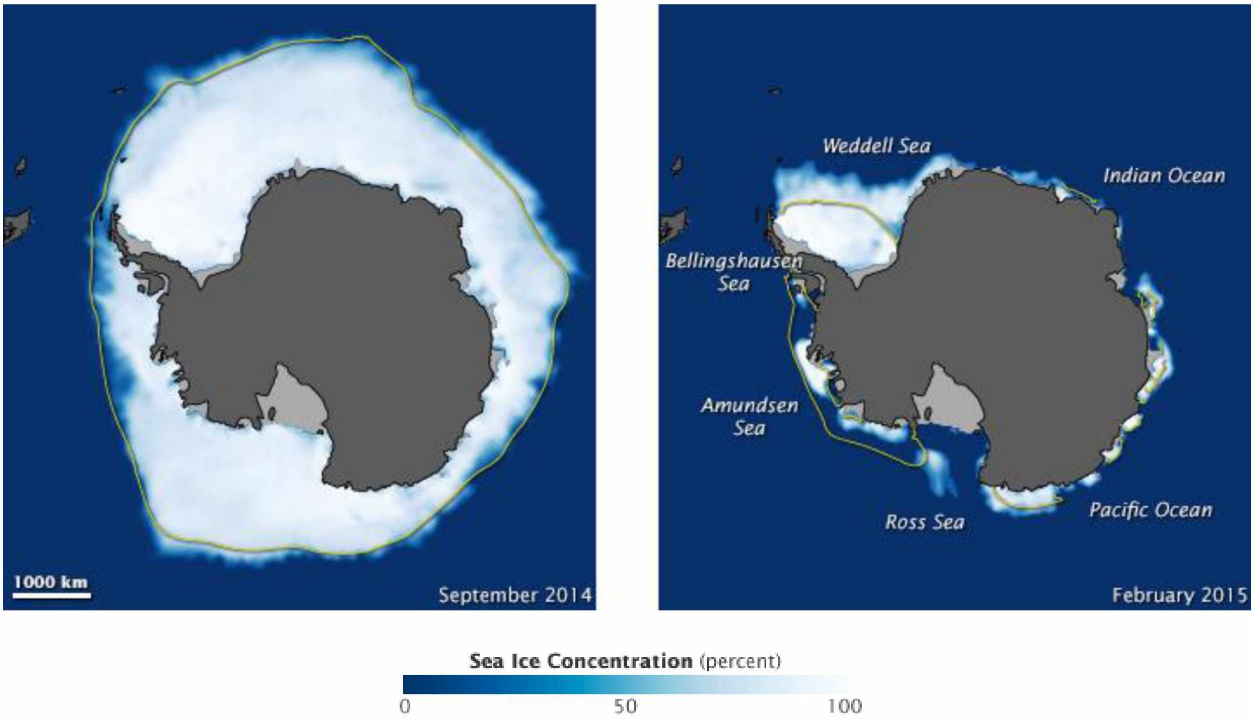

Figure 1.1. Annual sea ice extent (white) around the Antarctic continent. Maximum ice extent occurs in September, and minimum in February. Yellow outline indicates median ice extent from 1979-2000. Figure from the NASA Earth Observatory (http://earthobservatory.nasa.gov/Features/WorldOfChange/sea_ice_south.php).

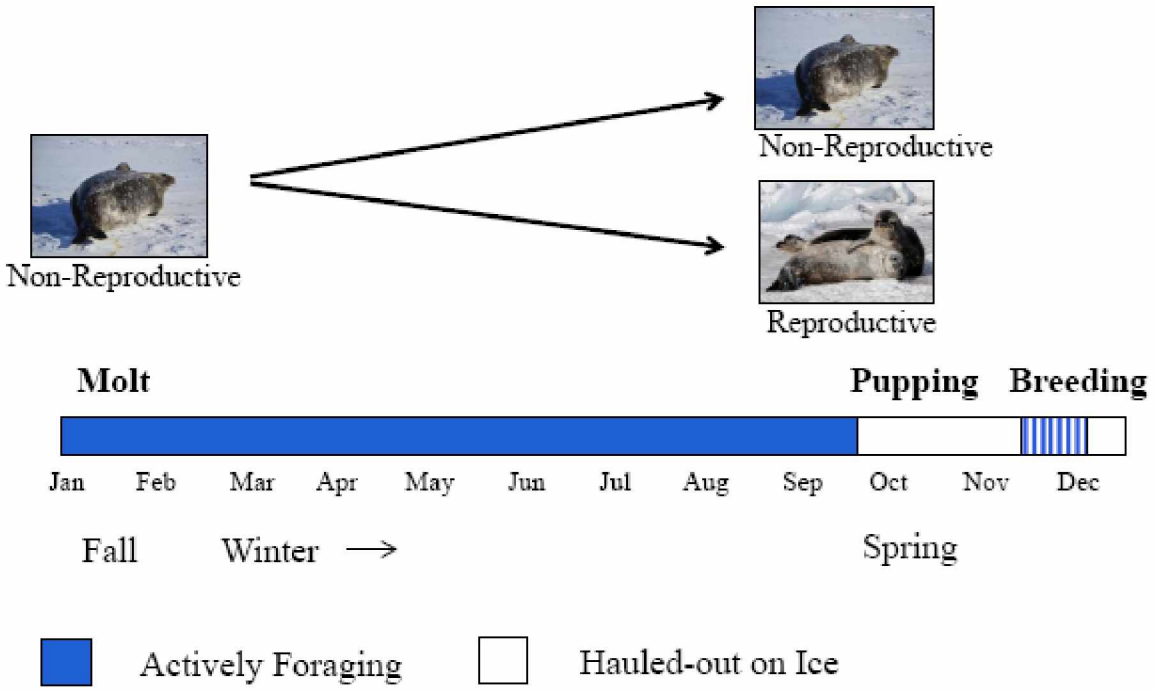

Figure 1.2. Annual life history cycle of the Weddell seal. *White* = times of the year that animals remain largely hauled-out on ice; *Blue* = animals actively forage. The dissertation study design utilizes non-reproductive females handled just after the annual molt and measures overwinter changes in physiology and dive behavior. Differences were assessed between females that returned the following year and gave birth, versus those that did not produce a pup.

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Chapter 2. Improving the Precision of Our Ecosystem Calipers: A Modified Morphometric Technique for Estimating Marine Mammal Mass and Body Composition[1]

2.1 Abstract

Mass and body composition are indices of overall animal health and energetic balance and are often used as indicators of resource availability in the environment. This study used morphometric models and isotopic dilution techniques, two commonly used methods in the marine mammal field, to assess body composition of Weddell seals (*Leptonychotes weddellii*, *N*=111). Findings indicated that traditional morphometric models that use a series of circular, truncated cones to calculate marine mammal blubber volume and mass overestimated the animal's measured body mass by 26.9 ± 1.5% SE. However, we developed a new morphometric model that uses elliptical truncated cones, and estimates mass with only -2.8 ± 1.7% error (*N*=10). Because this elliptical truncated cone model can estimate body mass without the need for additional correction factors, it has the potential to be a broadly applicable method in marine mammal species. While using elliptical truncated cones yielded significantly smaller blubber mass estimates than circular cones (10.2 ± 0.8% difference; or 3.5 ± 0.3% total body mass), both truncated cone models significantly underestimated total body lipid content as compared to isotopic dilution results, suggesting that animals have substantial internal lipid stores (*N*=76). Multiple linear regressions were used to determine the minimum number of morphometric measurements needed to reliably estimate animal mass and body composition so that future animal handling times could be reduced. Reduced models estimated body mass and lipid mass with reasonable accuracy using fewer than five morphometric measurements (root-mean-square-error: 4.91% for body mass, 10.90% for lipid mass, and 10.43% for %lipid). This indicates that when test datasets are available to create calibration coefficients, regression models also offer a way to improve body mass and condition estimates in situations where animal handling times must be short and efficient.

[1] Shero, M.R., L.E. Pearson, D.P. Costa, and J.M. Burns. 2014. Improving the precision of our ecosystem calipers: A modified morphometric technique for estimating marine mammal mass and body composition. PLoS One 9(3): e91233. DOI: 10.1371/journal.pone.0091233.

2.2 Introduction

Establishing links among variations in environmental conditions, prey availability, foraging success, and population status has become increasingly important as ecosystems face climate and anthropogenic threats. While monitoring ecosystem processes can be difficult, changes in the mass and body condition of apex predators can be used as indices of ecosystem health [1–5]. Accurate estimates of body mass and condition are also essential for a wide range of ecological and physiological studies, as they represent animals' net energetic costs or gains [6–8]. In addition to being a proxy for overall animal health and fitness, in marine mammals, body composition also influences animal streamlining, buoyancy, metabolic demand, and thermoregulatory costs [9–14].

In fieldwork situations, mass and body composition (e.g., lipid stores) can be most directly measured by weighing animals and using hydrogen-isotope dilution techniques, respectively. Isotopic dilution methods measure the animal's total body water (TBW) volume by allowing a bolus of labeled water to dilute within the body water pool. Measured TBW volume, coupled with estimates of the hydration state of lean tissue (73% water) and adipose (10% water), allows for relatively accurate (mean error: 3.7%) estimates of body composition in mammals [7,15,16]. Errors arise from the generation of metabolic water, exchange of isotope with non-aqueous hydrogen ions, dilution in stomach water, and evaporative water loss [6,16,17]. Method accuracy is also influenced by errors in the assumed hydration state of body tissue, as water content in the blubber and lean tissue may differ by species, season, and age [18–20]. Additionally, validations of isotopic dilution to true TBW and lipid stores by desiccation and dissection comparisons have only been performed in a select number of studies because these destructive methods are so labor intensive [21–25]. Still, isotopic dilution methods have been used in a wide range of species, including pinnipeds, and are generally assumed to be the "golden standard." Despite the potential sources of error, isotopic dilution has the advantage of accounting for both the subcutaneous and internal lipid stores, and in most study systems, body composition determined by these methods is considered to be the most reliable field measure of total body lipid content [6,20].

However, isotopic dilution protocols can be logistically difficult, costly, and time consuming. As a result, a wide variety of proxy variables have been identified to serve as indicators of marine

mammal body condition. Because marine mammals store large amounts of energy in their large subcutaneous blubber layer, these simpler methods have placed a large emphasis on blubber volume. Proxies range from models using a single length and girth measurement to estimate body mass, to a single blubber depth or bioelectrical impedance analysis to indicate animal body condition [25–28]. As these overly simplistic models are often poor predictors of body composition, Gales & Burton [29] outlined a technique for determining blubber volume in pinnipeds. Using morphometric (lengths and girths) and ultrasound blubber depth measurements, the animal's body shape is reconstructed as a series of circular truncated cones. Blubber and core tissue density estimates are then used to convert the calculated volumes of the cones to blubber mass. This truncated cone method has been used to determine blubber mass in multiple pinniped [8,9,11,30,31] and cetacean species [32,33]. However, soon after this method was developed, Slip et al. [34] described the phenomenon of "fat slumping" wherein gravity causes blubber along the lateral sides of the animal to "slump" and, therefore, causes the animal's true shape to deviate from circular towards elliptical.

Since Gales & Burton [29], many studies have used modeled estimates of blubber volume to calculate body composition, despite the fact that blubber mass measurements are not equivalent to lipid mass. Adult marine mammal blubber contains structural proteins and is not composed entirely of lipid. Blubber lipid content ranges from ~30-95%, depending on species, reproductive status, overall health, and season [19,35–38]. Still, very few studies incorporate actual blubber lipid content into calculations [31,39]. In addition, pinniped studies that solely use the truncated cones method as a measure of condition cannot account for internal lipid stores. Thus, potential sources of error should be acknowledged when evaluating the success with which a morphometric technique can determine body mass and condition.

However, morphometric models are so attractive because studies at the population level require large sample sizes to detect potentially small but significant changes in mass and condition. For large animals such as marine mammals, determining body mass via direct weighing is difficult, and isotopic dilution requires long sedation and equilibration times. Morphometric models thus offer a good alternative, yet very few studies have attempted to construct predictive models that employ only a few non-invasive measurements to estimate body mass and condition [34,40].

This study compared methods of estimating Weddell seal (*Leptonychotes weddellii*) body composition, including morphometric models and isotopic dilution techniques. In addition, we developed a modified truncated cone method that accounts for blubber and core body slumping by modeling animal cross-sectional shape as ellipses instead of circles, and compared accuracy of body mass and condition estimates. Then, we developed models to estimate body mass and composition from a few non-invasive morphometric measurements. Our findings provide a quantitative basis for choosing efficient and logistically feasible methods of assessing marine mammal body mass and condition under constrained field conditions.

2.3 Methods

2.3.1 Ethics Statement

Animal handling protocols were approved by the University of Alaska Anchorage and University of California Santa Cruz's Institutional Animal Care and Use Committees. Research and sample import to the United States was authorized under the Marine Mammal permit No. 87-1851-04 issued by the Office of Protected Resources, National Marine Fisheries Service. Research activities were approved through Antarctic Conservation Act permits while at McMurdo Station.

2.3.2 Animal Capture

Adult Weddell seals (*N*=111; Table 2.1) were captured on fast-ice in Erebus Bay (~77°S, 165°E) and the Victoria Land coastline (~76°S, 162°E), Antarctica from 2010-2012. Animals were handled in Jan/Feb (Austral fall) after the molt period when seals are typically in their poorest condition (lowest lipid stores) and in Oct/Nov (Austral spring; pre-breeding period) after the animals have been actively foraging for ~8 months [41].

Animals were sedated with an initial intramuscular dose of 1.0 $mg \cdot kg^{-1}$ tiletamine/zolazepam HCl, followed by intravenous injections of ketamine and diazepam (1:1 ratio; 100 $mg \cdot mL^{-1}$ and 5 $mg \cdot mL^{-1}$) as necessary.

2.3.3 Direct Measures of Total Body Mass & Lipid Stores

Total body mass (M_T) was determined by direct weighing (MSI-7200-1T Dyna-Link digital dynamometer, capacity 1,000 ± 1.0 kg).

The body composition of 76 animals (Table 2.1) was determined by isotopic dilution. Following collection of an initial blood sample, 1-1.5 mCi tritiated water (HTO) was injected into the extradural vein. Each syringe was gravimetrically calibrated prior to use, and syringes were flushed with blood after injection to ensure complete administration of the dose. Blood was collected from the extradural vein in serum separator vacutainers at 15-30 min intervals for 90-120 min post-injection. Serum was separated from whole blood samples via centrifugation and stored at -80°C until analysis. Water was extracted from samples in triplicate using the freeze-capture technique as described in Ortiz et al. [15]. HTO specific activity (counts per minute; CPM) was determined using a Packard Tri-Carb 2900TR liquid scintillation counter (Packard Bioscience Co., Meriden, CT) by adding 100 µL distillate into 10 mL ScintiSafe scintillation cocktail (Fisher Scientific, Inc.). Each of the triplicate distillate samples was counted twice for 20 min (~10,000-20,000 total counts). Triplicates were only accepted if CV's were < 2%. Pre-injection activity determined for each animal was subtracted from all post-injection activities. Injectate standards were distilled in six replicates before and after analyzing animal serum samples to ensure minimal intra-assay variation. Dilution curves plateaued by 90 min (Fig. 2.1), and total body water (TBW) was calculated as:

$$\text{(Eq. 2.1)} \qquad \text{TBW(kg)} = \frac{\text{activity of injected isotope}}{\text{activity of post equilibrated sample}}$$

TBW values were reduced by 3.3% to account for post-injection isotope losses due to exchanging hydrogen ions and ventilation [42]. Total body lipid mass (TBL_{HTO}) was calculated from TBW following Reilly & Fedak [24]:

$$\text{(Eq. 2.2)} \qquad \text{TBL}_{\text{HTO}}\text{(kg)} = 105.1 - \left[1.47 \times \left(\frac{\text{TBW}}{M_T}\right) \times 100\right]$$

where M_T is total body mass, and both TBW and M_T are in kg. Isotopic dilution techniques yielded total lipid mass, regardless of its location subcutaneously or internally, and lean mass was considered to be fat free tissue.

2.3.4 Truncated Cone Estimates of Body Mass & Composition

After animals were captured and weighed, a series of morphometric measurements were taken in order to model mass and body composition: girths, straight lengths (sLengths), and curvilinear lengths (cLengths) measured from the animal's nose to eight consecutive sites along the body (Fig. 2.2A). Subcutaneous blubber thickness was measured at six dorsal and lateral sites (Fig. 2.2A) using a SonoSite Vet180Plus portable ultrasound and 3.5 MHz convex transducer (SonoSite Inc., Bothell, Washington, USA) while the animal was in sternal recumbency. Blubber depth measurements were used to calculate blubber volume and mass using the traditional truncated cones method as described by Gales & Burton [29]. The animal's body shape was reconstructed as a series of circular truncated cones, and blubber volume was calculated as the volume of the outer cone (total body volume) minus the volume of the inner cone (core body volume; Fig. 2.2B). The volume of the head and tail were estimated using full cones composed entirely of lean mass, while flippers were not included in truncated cone models (Table 2.2) because there is very little blubber around the head, tail, fore-, and hind-flippers [43].

Because seals did not appear circular in cross-section when lying on the ice, the procedure was adjusted to include measurements of animal height and width at each site along the body for both outer and inner body cones (N=11). The circular truncated cones calculations [29] were then modified for elliptical body cross-sections in animals for which all measurements were taken (N=10; Fig. 2.2B; Table 2.3). In the modified elliptical cone method, the straight length for each truncated cone segment was calculated by using right triangles along the animal (Fig. 2.2A), with the measured curvilinear length as the hypotenuse and half the height difference between cone segments as the adjacent side of each triangle:

(Eq. 2.3) $$\text{Truncated cone segment sLength (cm)} = \sqrt{\left(\Delta\text{cLength}\right)^2 - \left(\Delta\frac{1}{2}\text{H}\right)^2}$$

where ΔcLength is the curvilinear length of the elliptical truncated cone segment and Δ½H is the height difference from the center of the frustum (half the animal height) for that segment of the animal. The volume of the total body outer elliptical truncated cone was calculated as:

$$\text{(Eq. 2.4)} \quad \text{Volume outer elliptical cone (L)} = \frac{\text{sLength} \times \pi}{12} \times \left(D_1 D_2 + D_3 D_4 + \sqrt{D_1 D_2 D_3 D_4} \right)$$

where D_1 and D_2 are the major (measured animal width) and minor (height) diameters of the anterior end of the cone segment, and D_3 and D_4 are the major and minor diameters of the posterior end of the cone segment, respectively. The summation of these elliptical truncated cone segments yielded total body volume. To determine the volume of the animal's inner core, blubber depths were subtracted from the body diameter:

$$\text{(Eq. 2.5)} \quad D_{1,\,\text{inner}} = D_1 - \left(2 \times \text{Blubber depth}_{\text{lateral}} \right) \text{ at the anterior end}$$

$$\text{(Eq. 2.6)} \quad D_{2,\,\text{inner}} = D_2 \quad \left(2 \quad \text{Blubber depth}_{\text{dorsal}} \right) \text{ at the anterior end.}$$

The same equations were used to find $D_{3,\,\text{inner}}$ and $D_{4,\,\text{inner}}$ at the posterior end of the cone segment. Once blubber depths were subtracted, Eqn (2.4) was used to calculate the volume of the inner core for each truncated cone segment using these modified diameters. Summation of the core truncated cones yielded core body volume, and blubber volume was calculated as the difference between the outer and inner core volumes:

$$\text{(Eq. 2.7)} \quad \text{Blubber Volume}_{\text{ellipse}}\text{(L)} = \text{Volume outer cone}_{\text{ellipse}} - \text{Volume inner core cone}_{\text{ellipse}}.$$

In both circular and elliptical truncated cone models, blubber and core volume estimates were converted to body mass by assuming that the lean body core and blubber layer had densities of 1.1 $g \cdot mL^{-1}$ and 0.94 $g \cdot mL^{-1}$, respectively [29,30,44]. Blubber and total body mass were estimated by summing the mass of each truncated cone segment, and the head and tail cones.

Blubber mass (BM) calculated using elliptical truncated cones (BM_E) was regressed against blubber mass estimated from traditional circular cones (BM_C). This relationship was highly significant, and the regression equation was used to estimate BM_E for animals where it could not be directly calculated.

2.3.5 Blubber Lipid Content

A blubber biopsy was taken from each animal after the site was prepped with Betadine and Lidocaine, using a sterile 6-mm biopsy punch just below the midline at the umbilicus (Fig. 2.2A), flash frozen and stored at -80°C. To compare lipid content from the single biopsy site to average values across the body, blubber was collected opportunistically from a female that died of natural causes in McMurdo Sound, < 24 hrs post-mortem, from all 12 sites where blubber depth was measured for truncated cone models.

Full thickness blubber biopsies were weighed to the nearest 0.001 g and lipid content of the samples was determined gravimetrically after extracting lipids using a 2:1 chloroform-methanol rinse in a Soxhlet apparatus [45,46]. In the event that blubber lipid content was not available for a particular animal, the average lipid content for that season and reproductive class was used to convert BM to lipid mass (Table 2.1). Lipid mass within the blubber layer (BLM) was determined for elliptical (BLM_E) and circular truncated cones (BLM_C) using BM determined by morphometric models, as described above, and the lipid content per unit mass in the blubber biopsy:

(Eq. 2.8) $$BLM = BM \times \text{proportion lipid in blubber}$$

2.3.6 Statistical Analyses

Prior to statistical analyses, data were assessed for outliers and normality. Body composition data were normally distributed and between 20-80%, and thus were not arcsine transformed. Results are reported as mean ± SE. To determine whether animals were indeed elliptical in cross-section, width:height ratios were compared to that of a circle (width:height =1) using one-sample t-tests, while a two-way ANOVA was used to assess differences in the width:height ratio across the body, and between the inner and outer cones (N=11). Paired t-tests were used to determine whether total body mass estimates derived from the circular truncated cone method differed from actual body mass (M_T). Repeated-measures ANOVAs with Bonferroni post-hoc tests were used to determine whether mass and body composition estimates from both elliptical (N=10) and circular (N=76) truncated cone methods differed from the direct measurement of M_T or TBL_{HTO}.

Regression analyses were used to determine significant relationships between calculated BM or BLM, and TBL_{HTO} (N=76).

To create models that maximized the R^2 and minimized root-mean-square-error (RMSE; equivalent to standard deviation), forward stepwise multiple regression models were used to estimate body mass and condition. M_T was estimated from straight length (sLength) and the square of axillary girth (LG^2) measurements to compare models to the simplest published methods [26,27], and also estimated using the suite of lengths and girths measured during this study (N=111). Animals for which all lengths, girths, and blubber depths could be measured in addition to TBL_{HTO} (N=76) were used to create regression models relating morphometric measures to body composition. TBL_{HTO} was estimated with and without true M_T included in models.

Second-order Akaike information criterion (AICc) tests were implemented using the R "MuMIn" package to select the best models, incorporating the fewest number of parameters, as would be useful if animal handling times in the field are constrained. Variables were only added to models when the ΔAICc of the added parameter was ≥ 2. Season, sex, and reproductive status were added as categorical "dummy" variables. If a categorical variable was an important parameter in the model, it was added to all regressions as this would be a known parameter in a fieldwork situation. When incorporating parameters into predictive models, multicollinearity was assessed by variance inflation factors (VIF); all were less than 7. While lower than a VIF of 10, which is typically considered to be a concern [47,48], to further ensure that added parameters were not a spurious result of multicollinearity, RMSE of models was determined using k-fold cross-validations (with 10 folds) using the R "DAAG" package. Parameters were only added to the model when RMSE decreased. All analyses were conducted in R (v 2.15.2) and significance was assessed at the 95% level ($P<0.05$).

2.4 Results

2.4.1 Morphometric Estimates of Body Mass versus Weighing

Animals in this study varied widely in M_T (181-502 kg), TBW (97.6-253.0 kg; 40.5-56.5% M_T), and TBL_{HTO} (54.3-186.0 kg; 22.0-45.5% M_T) measurements (Table 2.1).

Animals were elliptical-shaped in cross-section as indicated by width:height ratios >1 at all eight sites along the body (Fig. 2.3; One-sample t-tests- ears: t_{10}=4.114, P=0.002; neck to ankles: all t_{10} > 10, all P<0.001). Width:height ratios differed by site along the body (Fig. 2.3; Two-Way ANOVA- $F_{7, 160}$= 20.010, P<0.001) and, once blubber depths were subtracted, the inner core cone had even greater width:height ratios as compared to the outer cones (Two-Way ANOVA- $F_{1, 160}$= 20.436, P<0.001). Larger animals and those in better condition were not more ellipsoid-shaped as compared to smaller seals (Multiple Regression- M_T: $F_{8, 10}$= 18.022, P=0.054, TBL_{HTO}: $F_{8, 9}$= 5.268, P=0.325).

Using standard published values for blubber and body core density, estimated M_T using truncated cone calculations with elliptical cross-sections were not significantly different from measured M_T (Fig. 2.4A; Subset Study: Repeated measures ANOVA- $F_{2,18}$= 167.442; Elliptical mean error from M_T: -11.9 ± 6.8 kg; -2.8 ± 1.7% (range: -8.9 to +7.1%), Bonferroni post hoc- P=0.340). Conversely, estimates using traditional circular cones were significantly higher than M_T directly determined by weighing animals (Fig. 2.4A; Subset Study: Circular mean error from M_T: 108.4 ± 6.8 kg; 26.3 ± 1.4% (range: +20.6 to +35.3%), Bonferroni post hoc- P<0.001; Fig. 2.4B; Full Study: Circular error from M_T: 81.0 ± 3.5 kg; 22.8 ± 0.6% (range: +11.3 to +42.2%), Paired t-test, t_{75}= -23.339, P<0.001).

As the use of ellipses yielded smaller M_T estimates, it also resulted in smaller blubber and core mass estimates. Core body mass estimated using elliptical truncated cones was significantly smaller than estimates using the traditional circular cones (66.2 ± 1.5 vs. 91.7 ± 1.2% M_T; Paired t-test, t_9= 19.981, P<0.001). The difference in core body mass between the two models was substantially greater than the difference in BM.

2.4.2 Morphometric versus Isotopic Dilution Estimates of Body Composition

Blubber lipid content determined via biopsy sample (Table 2.1; range: 61.1-97.4%) was used to convert BM to subcutaneous lipid mass (BLM) for each seal separately. Blubber lipid content at the dorsal umbilicus site was similar to the average blubber lipid content across the body (-2.37% error) in the necropsied seal. All BM_E, BLM_C, and BLM_E models yielded significantly smaller blubber/lipid masses as compared to isotopic dilution (Fig. 2.4C; Subset Study: Repeated measures ANOVA- $F_{1.8,16}$= 112.845, Bonferroni post-hoc- all P<0.001). The difference between TBL_{HTO} and BLM_E was 10.5 ± 0.8% body mass. BM_C did not differ from TBL_{HTO} in the study subset, but BM_C and BLM_C were both significantly lower than TBL_{HTO} in the full study (Fig. 2.4D; Full Study: Repeated measures ANOVA- $F_{1.5,110.6}$= 190.941, Bonferroni post-hoc- all P<0.001). BM_C was significantly positively correlated with BM_E (Fig. 2.5A; Subset Study: Regression- $F_{1,9}$= 182.2, P<0.001), and this relationship was used to predict BM_E for animals in which all measurements could not be taken.

Truncated cones calculations underestimated TBL_{HTO}; however, regression models allowed BM to estimate TBL_{HTO}. All regressions between morphometric cone models and TBL_{HTO} were highly significant and produced low error (RMSE). Regression errors were similar between elliptical and circular truncated cone models (Fig. 2.5B-D; Regression- BM_E: $F_{1,74}$= 351.8, P<0.001; RMSE= 14.76 kg, 12.38% TBL_{HTO}; BLM_E: $F_{1,74}$= 274.4, P<0.001, RMSE= 16.16 kg, 13.54% TBL_{HTO}; BM_C: $F_{1,74}$= 350.5, P<0.001, RMSE= 14.80 kg, 12.41% TBL_{HTO}; BLM_C: $F_{1,74}$= 281.1, P<0.001, RMSE= 16.03 kg, 13.44% TBL_{HTO}). However, the slope correcting BM_C to TBL_{HTO} was significantly < 1 (95% CI: 0.774-0.959) indicating that traditional circular truncated cones underestimated TBL_{HTO} to a greater extent in larger animals. All slopes relating BM_E, BLM_C, or BLM_E to TBL_{HTO} were not significantly different from 1. Adding season as a variable in regression models allowed for more accurate estimates of TBL_{HTO} from BM (BM_E: t_{season}= 2.422, P=0.018, R^2=0.839, RMSE= 14.25 kg, 11.94% TBL_{HTO}; BM_C: t_{season}= 2.444, P=0.017, R^2=0.839, RMSE= 14.28 kg, 11.97% TBL_{HTO}), but adding season did not improve fit between TBL_{HTO} and BLM (e.g., BLM_E or BLM_C).

2.4.3 Estimating Mass & Body Composition from Regression Models

In the absence of direct M_T measurements, the best single morphometric measurements to take in order to estimate M_T were sternum ($F_{1,109}$= 794.458, P<0.001, R^2=0.879) and middle girths ($F_{1,109}$= 790.550, P<0.001, R^2=0.879). Using either of these two girth measurements accounted for 8.8% more variance than using the axillary girth measurement alone to estimate M_T ($F_{1,109}$= 412.882, P<0.001, R^2=0.791). Adding length measurements to the multiple regression improved model fit and decreased the RMSE. The best model included sternum girth, cLength, middle girth, and sLength, and this estimated M_T with RMSE of 16.16 kg or 4.91% M_T (Table 2.4).

The best model for estimating absolute lipid mass (TBL_{HTO}) included M_T, season, and blubber depth at the middle dorsal site (Table 2.4; RMSE: 11.87 kg; 9.95% TBL_{HTO}). If M_T could not be determined in the field, the best model to estimate TBL_{HTO} included sternum girth, season, sternum lateral blubber depth, and cLength measurements (Table 2.4; RMSE: 13.00 kg; 10.90% TBL_{HTO}). The best predictor of TBL_{HTO} (as $\%M_T$) was season and blubber depth measured at the middle dorsal site (Table 2.4, RMSE: 3.54 $\%M_T$; 10.43% for $\%TBL_{HTO}$).

2.5 Discussion

This study demonstrated the efficacy of the modified elliptical truncated cone model to estimate M_T and TBL_{HTO}, and showed that a reduced set of non-invasive measurements can be used to estimate these parameters with high accuracy. The traditional truncated cones model using circular animal cross-sections significantly overestimated M_T and BM (absolute and as $\%M_T$), relative to elliptical cones. Still, both the circular and elliptical truncated cone models underestimated lipid stores as measured by using isotopic dilution techniques.

That body lipid stores determined by isotopic dilution techniques (TBL_{HTO}) are consistently higher than blubber lipid stores, both using elliptical and circular body cross-sections (BLM_E and BLM_C), suggests Weddell seals have significant internal lipid deposits that would be overlooked by solely using morphometric measures of condition. Previous work has demonstrated the presence of intramuscular lipid reserves and lipid sheaths around internal organs and abdominal mesentery [18,20,49–51]. These internal stores may be mobilized first during times of reduced

foraging [52], and would also impact the animal's net buoyancy and cost of locomotion [14,53]. Therefore, ignoring internal lipid reserves could introduce biases when comparing body composition among species, populations, and seasons.

The errors in Weddell seal M_T estimates using traditional circular truncated cones were not substantially improved when blubber and core tissue densities were slightly altered. Only if the total body average density of Weddell seals was assumed to be 0.83 ± 0.01 g·mL^{-1} were estimates of M_T equal to measured values. This density is well outside of the physiologically-relevant range, as measured blubber densities are 0.92-0.95 g·mL^{-1} [29,54], and the average densities of lean mass components for mammals are approximately 1.1 g·mL^{-1} [29,30,44,55]. In contrast, elliptical truncated cones provided estimates of M_T that were not significantly different from measured values when using published blubber and core density estimates to convert body volume to mass. Further, elliptical models did not require additional empirically-determined correction factors to accurately estimate M_T.

The fact that elliptical, but not circular, truncated cones closely approximated actual M_T indicates that a major source of error in the traditional truncated cones method is the assumption that animals are circular in cross-section. Elliptical cross-sections much more accurately reflect the animals' true body shape while hauled-out and lying flat against the ice. This deformation, or "slumping," was first described by Slip et al. [34], and was supported by the fact that field measurements of sculp mass were smaller than estimates using the traditional circular truncated cones calculations [56]. However, this study is the first, to our knowledge, to demonstrate that the compression of the core body mass into non-circular form introduces error. Using circular truncated cones to estimate core body mass produced values that were much too high, at 91.7% M_T. These errors arise because circles have the largest area per unit arc length, and therefore, the greater the degree of asymmetry of the animal (i.e., greater width:height ratio), the larger the overestimate in cross-sectional area.

The errors accompanying M_T and BM estimates from circular cones are likely to be important when calculating animal drag forces, buoyancy, density, and metabolic costs; all of which are influenced by surface area and volume calculations (Table 2.5). Conversely, variations between circular and elliptical models likely would not impact the relative differences and trends when

simply comparing body condition within a population. Thus, either method could be used as an ecosystem metric or index, provided it is understood that circular truncated cones are not yielding accurate estimates of mass or body composition unless additional correction factors are included in the model.

This study indicates that animal M_T and BM can be accurately estimated with volumetric and morphometric models, but that accurate estimates of lipid stores require isotopic dilution techniques or additional calibration factors. In combination, direct weighing and isotopic dilution techniques were found here to be the most appropriate tools when precise measures of animal size, body composition, or energetic costs are required. As this is not always possible in a field setting, there is a need for predictive models that relate proxy variables, such as morphometric measurements, to mass or body composition [34,40]. Such models do not make assumptions about animal shape, but instead rely on a "calibration dataset" for coefficient development. This study has shown that, once developed, these simplified models can be used to estimate mass and body composition quickly for a much larger sample size. Moreover, they can be quite accurate (RMSE: 4.91% for M_T; 10.90% for TBL_{HTO}; 10.43% for % TBL_{HTO}), and require much less time and effort as compared to direct measures or the more complex truncated cones models. Indeed, the multiple linear regression approach predicted body composition more accurately than the truncated cone models, while using far fewer morphometric measurements. Surprisingly, the models that produced the most accurate estimate of M_T included sternum rather than the axillary girth measure, which has traditionally been incorporated into the straight length × axillary girth^2 (LG^2) proxy [26,27]. Similarly, the model that produced the most accurate absolute and percent TBL_{HTO} used middle dorsal or sternum lateral blubber depth measures, respectively, instead of the traditional single axillary blubber measurement [28].

Identifying suitable proxy variables using hierarchical regressions can lead to reduced handling times and simpler procedures; however, predictive power will depend on model development using test datasets. This is because the relationship between morphometric measures with animal M_T and TBL_{HTO} are likely to be species-specific and vary seasonally. In contrast, the elliptical truncated cones method does not require such calibration coefficients and is, therefore, more broadly applicable and useful in new species and field situations. In addition to utilizing morphometric measurements to estimate M_T, there have been some recent successes in

photogrammetric methods. While these techniques to estimate M_T have been validated within <2-10% accuracy in pinnipeds and allow researchers to avoid animal handling [57–59], photogrammetry cannot quantify total body lipid stores. Alternatively, dive loggers have been used to estimate net animal buoyancy by measuring changes in animal drift rates through the water column. Since buoyancy is influenced by total body lipid content (both in the blubber and internal stores), drift rates are used as a proxy of changes in body composition [53,60,61]; however, this method does not provide estimates of M_T. The optimal technique for determining animal mass and body composition clearly depends on multiple factors such as handling and analytical constraints, whether precise or index values are needed to accomplish study goals, and the availability of test datasets for development of appropriate correction factors.

2.6 Acknowledgements

We thank field team members: Kimberly Goetz, Dr. Patrick Robinson, Dr. Luis Hückstädt, and Michelle LaRue for sample collection, group B-009-M led by Dr. Robert Garrott, Dr. Jay Rotella, and Dr. Thierry Chambert, and also group B-470-M led by Drs. Jo-Ann Mellish, Markus Horning, and Allyson Hindle for their help locating study animals. We also thank an anonymous reviewer whose suggestions improved this manuscript. Logistical support was provided by the National Science Foundation (NSF) U.S. Antarctic Program, Raytheon Polar Services, and Lockheed Martin ASC; we thank all the support staff in Christchurch, NZ and McMurdo Station. This research was conducted with support from NSF ANT-0838892 to D.P.C. and ANT-0838937 to J.M.B. This material is based upon work supported by the National Science Foundation Graduate Research Fellowship under Grant No. DGE-1242789. Any opinion, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation. M.R.S. and this research were supported in part by a UAF Center for Global Change Student Research Grant with funds from the Cooperative Institute for Alaska Research and the Alaska Climate Science Center. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. Conceived and designed the experiments: MRS JMB.

Performed the experiments: MRS JMB LEP DPC. Analyzed the data: MRS. Contributed reagents/materials/analysis tools: MRS JMB DPC. Wrote the paper: MRS JMB LEP DPC.

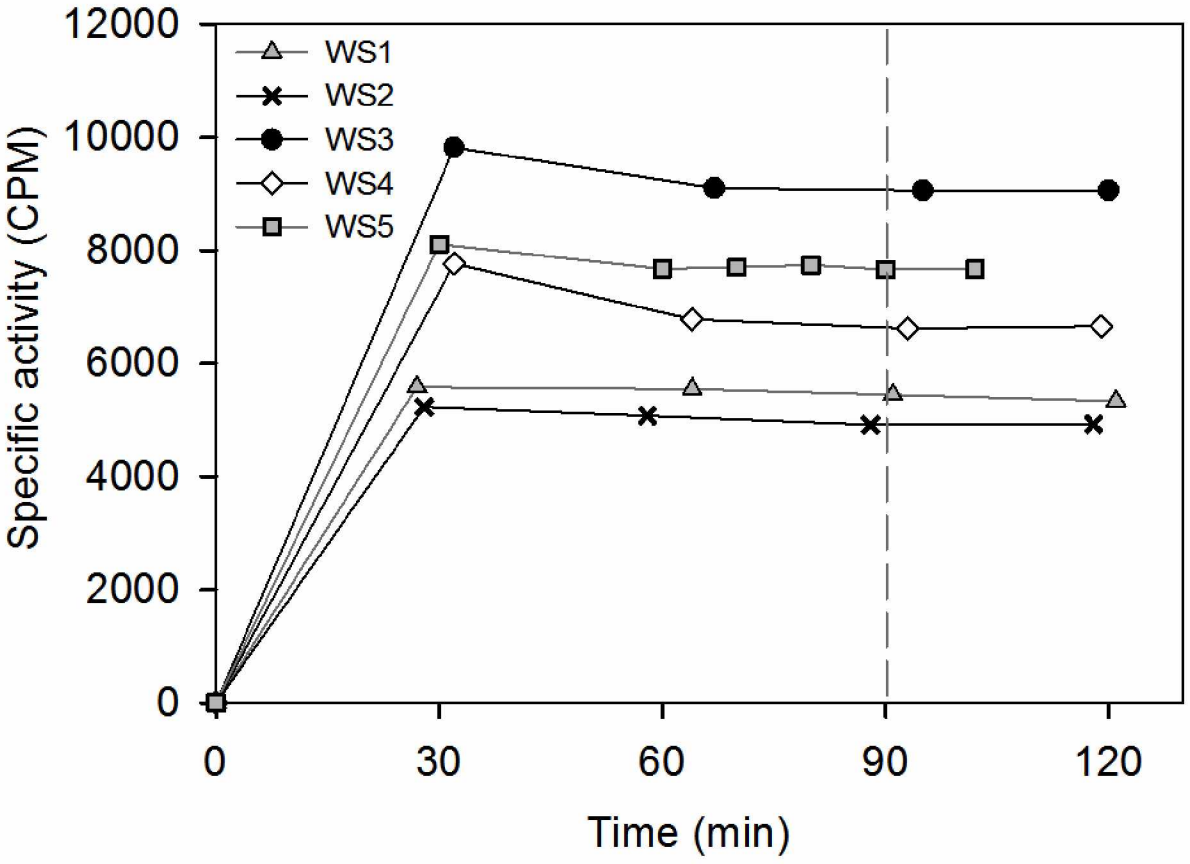

Figure 2.1. HTO equilibration curve for five Weddell seals showing plateau by 90 min.

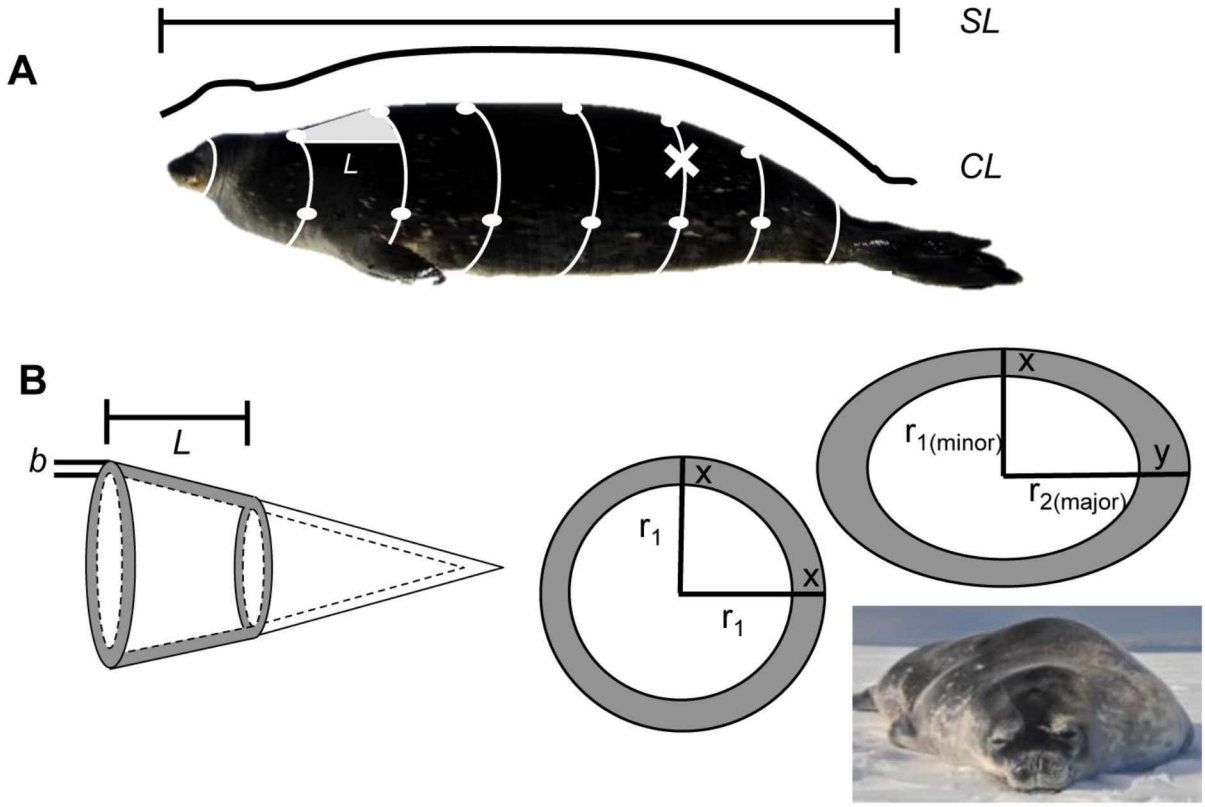

Figure 2.2. Morphometric measurements taken for each study animal. (**A**) *SL*=Standard length, *CL*=Curve length, Girths= white lines, Blubber depths= white dots, Cone section length calculations= grey triangle and "L". Site of blubber biopsy is marked with "X". (**B**) Reconstruction of truncated cones with segment length "*L*" and blubber depth "*b*" (*At left*). Circular and elliptical cross-sections shown (*At right*). Because an ellipse has a major and minor radius "r," the model can account for different dorsal and lateral blubber depths (x and y) and more accurately reflect true animal shape.

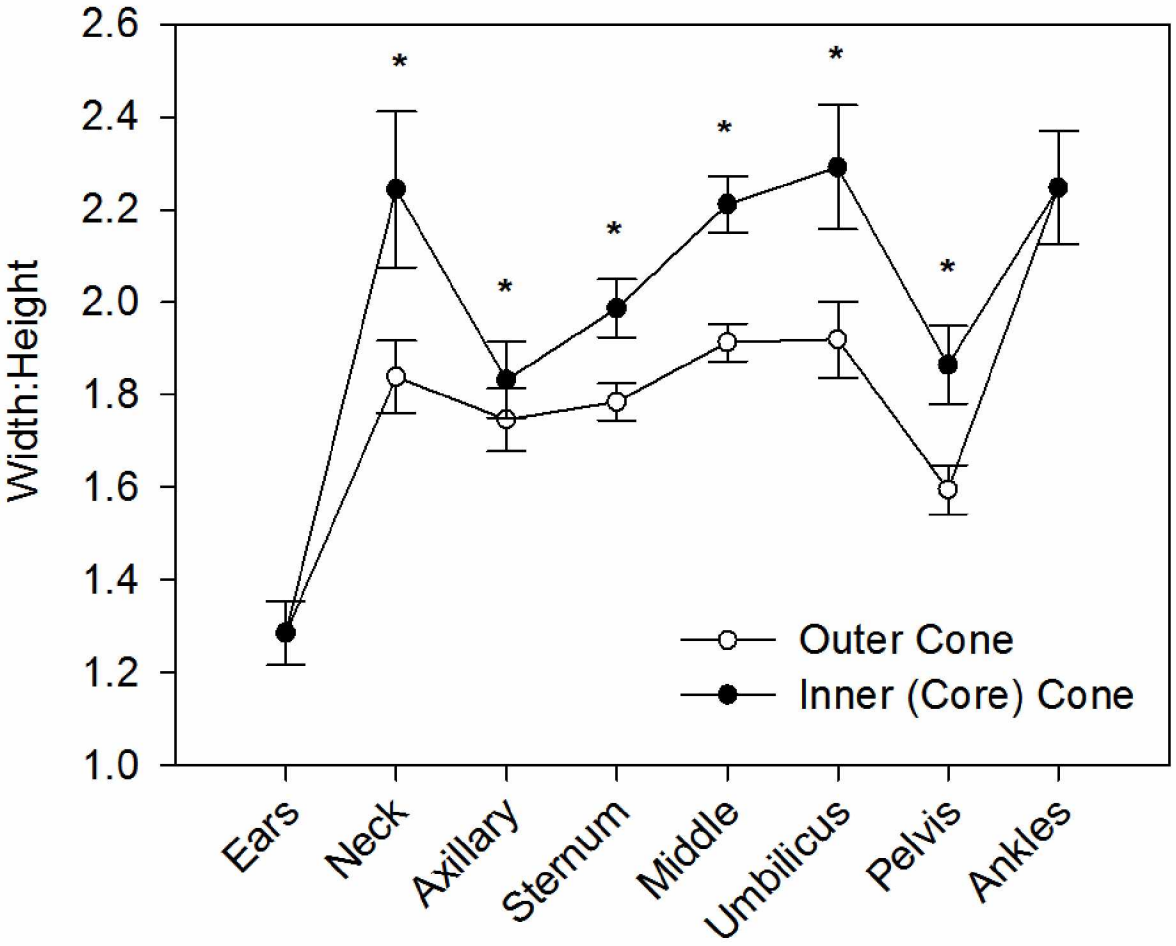

Figure 2.3. Weddell seal body cross-sections are elliptical. Mean ± SE width-to-height ratios along the body of adult female Weddell seals (*N*=11), with a circle having a ratio=1. *Asterisk* indicates that the width-to-height ratio of the inner core cone is significantly greater than the outer, total body cone.

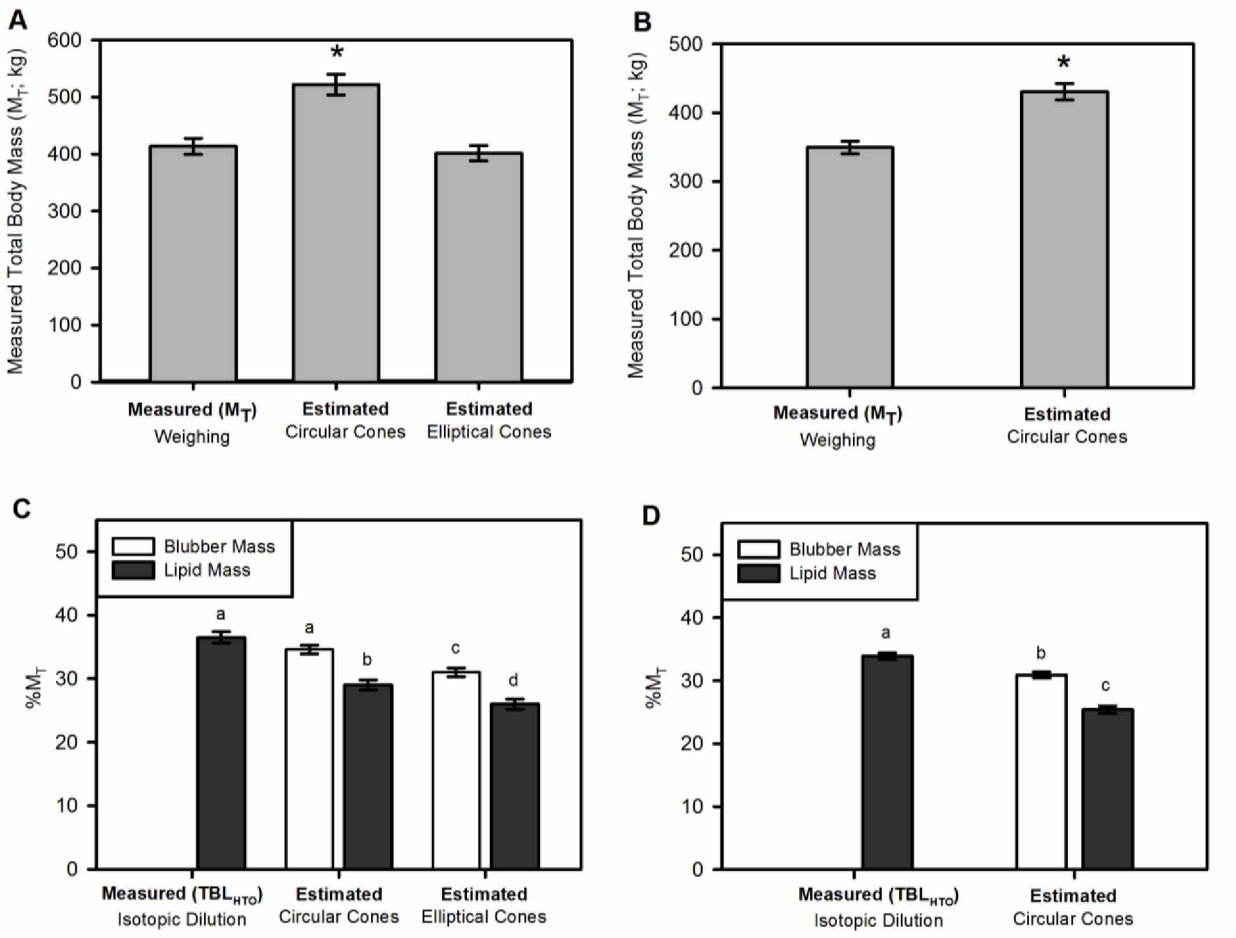

Figure 2.4. Estimated mass and body composition using truncated cones methods relative to measured values. Mean ± SE estimated total body mass (M_T) from the "subset study" (**A**; *N*=10) using both circular and elliptical truncated cones, and (**B**) from the "full study" (*N*=76) using circular truncated cones. Body composition estimated (**C**) from circular and elliptical cones in the subset study and (**D**) circular cones from the full study are also shown. Blubber with or without corrections for lipid content were compared to total body lipid determined via isotopic dilution (TBL_{HTO}). *= significant difference between estimated and measured M_T. Different letters= significant difference between body composition estimates relative to measured lipid stores.

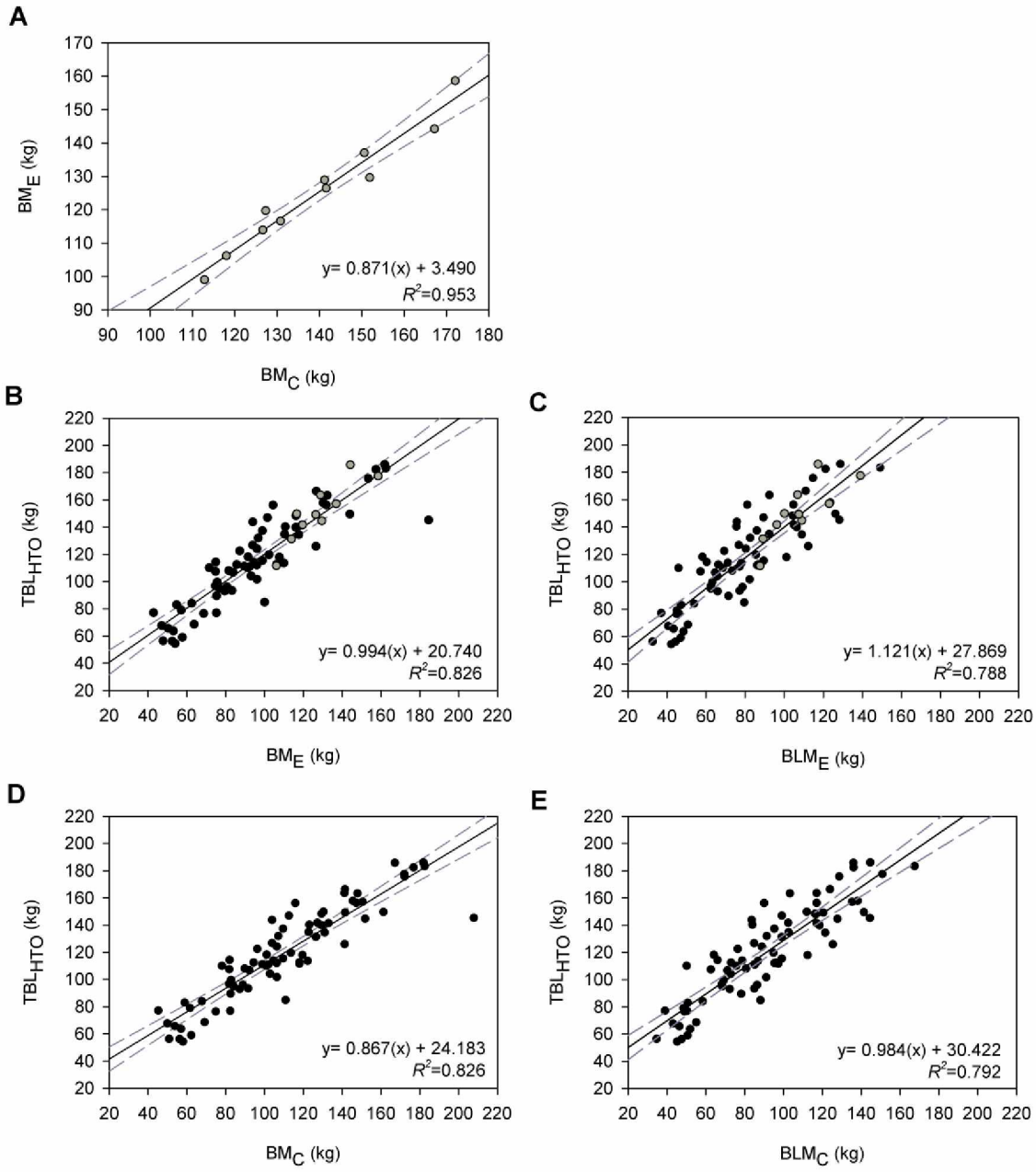

Figure 2.5. Relationships between morphometric and isotopic dilution body composition results. Linear regression between blubber mass determined using (**A**) elliptical and circular truncated cones (*N*=11). Once this relationship (*grey*) was used to correct values to elliptical models for additional animals (*black*), regressions were made between lipid mass determined by HTO measurements and elliptical cones with (**B**) and without (**C**) corrections for blubber lipid content. Similar relationships exist when using traditional, circular truncated cones with (**D**) and without (**E**) corrections for blubber lipid content (*N*=76).

Table 2.1. Sample sizes and means ± SE for body mass and composition.

Season	Reproductive Status	Total Body Mass (M_T; kg)	TBW (%M_T)	Lipid by HTO (%M_T)	Blubber Biopsy Lipid Content (%)
Jan/Feb	Skip-Breeding Female	320.7 ± 10.3 (*52*)	50.7 ± 0.5 (*32*)	30.5 ± 0.7 (*32*)	79.3 ± 1.3 (*49*)
	Male	231.8 ± 12.5 (*10*)	50.2 ± 0.7 (*7*)	31.4 ± 1.1 (*7*)	86.2 ± 2.2 (*5*)
Oct/Nov	Non-Reproductive Female	335.8 ± 14.2 (*28*)	45.9 ± 0.6 (*22*)	37.6 ± 0.8 (*22*)	83.5 ± 1.4 (*24*)
	Reproductive Female	413.7 ± 13.3 (*16*)	46.4 ± 0.6 (*13*)	36.8 ± 0.9 (*13*)	84.1 ± 1.9 (*17*)
	Male	294.6 ± 11.7 (*5*)	47.5 ± 1.2 (*2*)	35.4 ± 1.9 (*2*)	---
Overall	All	328.8 ± 7.6 (*111*)	48.5 ± 0.4 (*76*)	33.9 ± 0.6 (*76*)	81.6 ± 0.9 (*95*)

Mean ± SE total body mass (M_T), total body water (TBW) and lipid stores as determined by isotopic dilution (as %M_T), and lipid content of blubber biopsies (% wet mass) for animals handled throughout this study. Animals are classed by season and reproductive status, and sample sizes are in parentheses.

Table 2.2. Calculation of subcutaneous fat for seal WS12-22 using traditional truncated cones with circular cross-sections.

		Outer Cone Measurements					Inner Cone Measurements	Body Volume			Mass Conversions		
Cone Position		Girth (cm)	Radius (Outer; cm)	Radius Difference (cm)	Curvilinear Length (cm)	Straight Length (cm)	Average Blubber Depth (cm)	Radius (Inner; cm)	Outer Cone (L)	Inner Cone (L)	Blubber (L)	Core (kg)	Blubber (kg)	Total Body (kg)
1	**nose**	0	0	0	0	0	0	0	0	0	0	0	0	0
2	**nose → ears**	75	11.9	11.9	20	16.1	0	11.9	2.4	2.4	0	2.6	0	2.6
3	**ears → neck**	137	21.8	9.9	27	25.1	5.99	15.8	23.1	15.3	7.8	16.8	7.3	24.2
4	**neck → axillary**	202	32.2	10.4	37	35.5	6.13	26.0	82.2	49.8	32.4	54.8	30.5	85.3
5	**axillary → sternum**	121	33.7	1.6	31	31.0	5.64	28.1	105.6	71.3	34.3	78.4	32.3	110.7
6	**sternum → middle**	206	32.8	1.0	38	38.0	5.32	27.5	132.1	92.1	39.9	101.3	37.5	138.9
7	**middle → umbilicus**	181	28.8	4.0	37	36.8	5.48	23.3	109.8	74.7	35.1	82.2	33.0	115.1
8	**umbilicus → pelvis**	127	20.2	8.6	42	41.1	4.55	15.7	78.4	49.7	28.7	54.7	26.9	81.6
9	**pelvis → ankles**	76	12.1	8.1	23	21.5	0	12.1	18.0	13.1	4.9	14.4	4.6	19.0
10	**ankles → tail**	0	0	12.1	21	17.2	0	0	2.6	2.6	0	2.9	0	2.9
								Total:	**554.2**	**371.0**	**183.1**	**408.2**	**172.1**	**580.3**
											Measured Mass (kg):			**451.0**
											Error from Measured Mass:			**28.7%**

Radius outer cone=Girth/2π;
Radius difference=base − roof of cone;
Straight Length=sqrt(Curvilinear Length^2 − Radius Difference^2);
Radius inner cone=Radius outer cone – (2 × Blubber Depth);
Outer/Inner Cone Volume=(1/3)π × [(Radius Outer/Inner$_1$)^2 + (Radius Outer/Inner$_1$ × Radius Outer/Inner$_2$) + (Radius Outer/Inner$_2$)^2];
Blubber Volume= Outer Cone Volume − Inner Cone Volume;
Mass Conversions: Core Mass= Core Volume × 1.1; Blubber Mass= Blubber Volume × 0.94; Total Body Mass= Core Mass + Blubber Mass

Table 2.3. Calculation of subcutaneous fat for seal WS12-22 using truncated cones with modified, elliptical cross-sections.

		Outer Cone Measurements						Inner Cone Measurements				Body Volume			Mass Conversions		
Cone Position		Body Height (cm)	Body Width (cm)	Minor Radius (Outer Cone; cm)	Minor Radius Difference (cm)	Curvilinear Length (cm)	Straight Length (cm)	Dorsal Blubber Depth (cm)	Lateral Blubber Depth (cm)	Diameter Minor Axis (cm)	Diameter Major Axis (cm)	Outer Cone (L)	Inner Cone (L)	Blubber (L)	Core (kg)	Blubber (kg)	Total Body (kg)
1	nose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	nose → ears	20	33	10.0	10.0	20	17.3	0	0	20.0	33.0	3.0	3.0	0	3.3	0	3.3
3	ears → neck	27	57	13.5	3.5	27	26.8	5.25	6.72	16.5	43.6	22.5	14.5	8.0	15.9	7.5	23.4
4	neck → axillary	42	73	21.0	7.5	37	36.2	5.10	7.15	31.8	58.7	64.3	35.5	28.8	39.1	27.0	66.1
5	axillary → sternum	44	81	22.0	1.0	31	31.0	5.57	5.71	32.9	69.6	80.6	50.4	30.1	55.5	28.3	83.8
6	sternum → middle	41	85	20.5	1.5	38	38.0	5.82	4.82	29.4	75.4	105.1	67.1	38.0	73.8	35.7	109.5
7	middle → umbilicus	34	68	17.0	3.5	37	36.8	5.68	5.28	22.6	57.4	83.3	50.2	33.0	55.3	31.1	86.3
8	umbilicus → pelvis	30	45	15.0	2.0	42	42.0	4.67	4.42	20.7	36.2	59.6	33.3	26.3	36.6	24.7	61.4
9	pelvis → ankles	12	35	6.0	9.0	23	21.2	0	0	12.0	35.0	14.0	9.6	4.4	10.5	4.1	14.7
10	ankles → tail	0	0	0	6.0	21	20.1	0	0	0	0	2.2	2.2	0	2.4	0	2.4
											Total:	**434.6**	**265.8**	**168.6**	**292.4**	**158.6**	**451.0**
													Measured Mass (kg):				**451.0**
													Error from Measured Mass:				**0.0%**

Minor radius outer cone=Body Height/2;
Minor radius difference=base − roof of cone minor radius;
Straight Length=sqrt(Curvilinear Length^2 – Minor Radius Difference^2);
Diameter inner cone major/minor axis= Body Height/Width – (2 × Blubber Depth);
Outer/Inner Cone Volume= [(Straight length × π)/12] × [$D_1D_2 + D_3D_4$ + sqrt($D_1D_2D_3D_4$)];
Blubber Volume= Outer Cone Volume − Inner Cone Volume;
Mass Conversions: Core Mass= Core Volume × 1.1; Blubber Mass= Blubber Volume × 0.94; Total Body Mass= Core Mass + Blubber Mass

Table 2.4. Morphometric measurements to estimate body mass and composition.

Estimated Parameter	Equation	AICc	ΔAICc	R^2	RMSE; kg (%M_T)
Mass (kg)	4.676×10^{-5} (sLength × Girth:axillary^2) - 11.399	1043.26	11.98	0.896	26.57 (8.08)
Traditional LG^2 only	4.553×10^{-5} (sLength × Girth:axillary^2) + 18.442(Season) - 10.642	1031.28	0	0.908	25.14 (7.65)
	Equation	**AICc**	**ΔAICc**	**R^2**	**RMSE; kg (%M_T)**
Mass (kg)	4.398(Girth:sternum) - 468.287	1059.47	125.10	0.879	28.34 (8.62)
All morphs	3.003(Girth:sternum) + 1.589(cLength) - 613.603	988.98	54.65	0.937	20.62 (6.27)
	1.443(Girth:sternum) + 1.420(cLength) + 1.565(Girth:middle) - 565.268 *	939.14	4.81	0.961	16.55 (5.03)
	1.509(Girth:sternum) + 0.985(cLength) + 1.497(Girth:middle) + 0.534(sLength) - 580.934 *	934.33	0	0.963	16.16 (4.91)
	Equation	**AICc**	**ΔAICc**	**R^2**	**RMSE; kg (%TBL_{HTO})**
TBL_{HTO} (kg)	0.388(M_T) - 16.258	638.65	44.71	0.793	15.81 (13.25)
M_T included	0.349(M_T) + 20.798(Season) - 12.840	600.6	6.66	0.878	12.29 (10.30)
	0.300(M_T) + 16.327(Season) + 6.485(Blubb:middle dorsal) - 21.621	593.94	0	0.892	11.87 (9.95)
	Equation	**AICc**	**ΔAICc**	**R^2**	**RMSE; kg (%TBL_{HTO})**
TBL_{HTO} (kg)	1.799(Girth:sternum) - 213.980	646.33	38.28	0.771	16.70 (14.00)
M_T not included	1.609(Girth:sternum) + 18.227(Season) - 187.788	624.13	16.08	0.834	14.32 (12.00)
	1.305(Girth:sternum) + 8.406(Season) + 8.770(Blubb:sternum lateral) - 165.120	615.25	7.20	0.857	13.82 (11.58)
	0.955(Girth:sternum) + 11.712(Season) + 8.334(Blubb:sternum lateral) + 0.373(cLength) - 195.099	608.05	0	0.874	13.00 (10.90)
	Equation	**AICc**	**ΔAICc**	**R^2**	**RMSE; %M_T (%TBL_{HTO})**
TBL_{HTO} (%M_T)	5.152(Season) + 1.287(Blubb:middle dorsal) + 25.749	408.53	0	0.508	3.54 (10.43)

Stepwise forward multiple regressions using morphometric measurements to estimate total body mass (M_T) and lipid mass (TBL_{HTO}; absolute kg and as %M_T). Factors that were included in each model are shown under the estimated parameter. Each step is shown to elucidate which measurements should be taken preferentially, if animal handling time is limited (all $P<0.001$). *= Note that the additional parameter in this model had slightly increased the variance inflation factor, and the variance in the coefficients. All lengths, girths, and blubber depths were measured in cm, and when season is a significant parameter, the coefficient should be multiplied by "0" for January and "1" for October study animals. Root-square-mean-error (RMSE) of models is presented as absolute (kg) and as a percentage of the study's mean M_T or TBL_{HTO}.

Table 2.5. Differences in additional physiological parameters determined by circular vs. elliptical truncated cones.

Physiological Estimate	Circular Cones	Elliptical Cones
Total Volume (L)*	490.3 ± 17.3	379.8 ± 13.4
Surface Area (SA; cm^2)	3773.4 ± 93.2	3773.4 ± 93.2
SA:V*	7.73 ± 0.10	9.98 ± 0.15
Net Buoyancy (N)*	-57.3 ± 5.0	-76.2 ± 5.7
Calculated Density ($g \cdot mL^{-1}$)*	0.83 ± 0.01	1.07 ± 0.02

Mean ± SE total body volume, surface area, surface area-to-volume ratios, buoyant force, and calculated density in Weddell seals (N=11). Surface area remains the same between circular and elliptical cones. Buoyancy was calculated following Webb et al. [9], and density was calculated using measured M_T. *Asterisk* indicates a significant difference in estimated parameter using circular versus elliptical models (paired t-tests; all P<0.001).

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Chapter 3. How Do Overwinter Changes in Body Condition and Hormone Profiles Influence Weddell Seal Reproductive Success?[1]

3.1 Abstract

1. Reproductive success can be influenced by maternal physiological condition at the time of embryo implantation and by foraging success during gestation. Polar marine mammals experience drastic fluctuations in body composition (lipid stores) as a result of life history events and large-scale changes in seasonal productivity and environmental conditions. These species provide the opportunity to explore physiological parameters important to reproductive success.

2. There are conflicting physiological demands on Weddell seal (*Leptonychotes weddellii*) females during the moult period, when animals are at their leanest but still must generate an energetically-costly new pelage and begin active gestation.

3. To investigate the impact of post-moult condition and hormonal mediators on the reproductive success of the southernmost breeding mammal, body composition was determined for post-moult (fall; 53 non-reproductive) and pre-breeding (spring; 31 non-reproductive, 17 reproductive) adult female Weddell seals. Animals were significantly larger and had greater lipid stores in spring, after the winter foraging period. There were no differences in the proportion of mass or condition gained overwinter between females that gave birth (n= 12) and those that did not (n= 8) the following year.

4. Changes in body condition were correlated with endocrine factors that influence energy allocation, such as cortisol, growth hormone (GH), insulin-like growth factor (IGF)-1, and thyroid hormones (T_3 and T_4). Of these, GH and T_4 were significantly higher during the post-moult period, likely to promote protein sparing and hair regeneration. In

[1] Shero, M.R., R.T. Krotz, D.P. Costa, J.P. Avery, and J.M. Burns. 2015. How do overwinter changes in body condition and hormone profiles influence Weddell seal reproductive success?. Functional Ecology DOI: 10.1111/1365-2435.12434.

addition, females that had higher T_4 concentrations in fall were significantly more likely to have a pup the following year, possibly due to the role of thyroid hormones in embryo attachment. This suggests that hormones influencing fuel use during the moult may also impact subsequent reproductive success.

5. Unlike some other large pinnipeds, Weddell seals are not capital breeders. This work indicates that gestating Weddell seals do not gain as much mass or energy overwinter in preparation for lactation the following year as lower-latitude phocid species, which might explain why female Weddell seals rely on foraging to meet energetic demands during lactation.

3.2 Introduction

The survival and reproductive output of wild animal populations hinges on the individual's ability to withstand the daily, seasonal, and annual variation in its habitat and resources. Changes in body mass and condition are standard metrics to assess energy acquisition relative to expenditure when flux cannot be directly measured (Costa et al. 1986; Crocker et al. 2001; Speakman 2001; Thordarson, Vikingsson & Hersteinsson 2007; Castellini et al. 2009). During times when resources are abundant, animals can accumulate endogenous energy stores in order to buffer the negative impacts of future resource limitations. Therefore, changes in mass and lipid stores are often used as a proxy for foraging success and fitness (Bowen et al. 2001; Crocker et al. 2001; McDonald et al. 2008).

In marine mammals, energetically-costly critical life history events involve complex physiological and behavioural shifts. The Weddell seal, *Leptonychotes weddellii*, is the southernmost living mammal and hauls out on fast-ice anchored to the Antarctic continent to give birth in the austral spring. Females cease foraging for the first days to weeks post-partum, but towards the end of the lactation period they spend up to 25% of the day diving (Hindell et al. 2002, Sato et al. 2002). Adult females lose approximately 30% of their body mass while pups double or triple in size across the 6-7 week lactation period (Fenwick 1973; Castellini, Davis & Kooyman 1992; Wheatley et al. 2006). For post-partum females, breeding and moult follow in

quick succession after weaning (Stirling 1969; Fenwick 1973; Worthy et al. 1992). The need to reduce foraging time during the moult so that the skin can remain warm and hair regrowth can proceed imposes additional nutritional constraints (Feltz & Fay 1966; Castellini et al. 1992; Boily 1995). Numerous phocid species mobilize lipid from their subcutaneous blubber layer to offset these times of negative energy balance (Costa et al. 1986; Castellini & Rea 1992; Oftedal 1993; Bowen et al. 2001; Wheatley et al. 2006; McDonald et al. 2008), and some catabolism of endogenous protein is required during lactation and the moult (Castellini & Rea 1992; Noren et al. 2003). Weddell seals then forage intensively over the austral winter (Castellini et al. 1992; Testa 1994; Schreer & Testa 1996) both to sustain the cost of gestation and to acquire sufficient reserves for the next year's reproductive events (Carlini et al. 1999; Beck, Bowen & Iverson 2003; McDonald et al. 2008; Robinson et al. 2012).

Changes in body condition and metabolic hormones during the annual cycle of natural fasting in pinnipeds have been well documented (Guinet et al. 2004; Kumagai, Rosen & Trites 2006; Rosen & Kumagai 2008; Champagne et al. 2012a; Champagne et al. 2012b; Crocker et al. 2012; Crocker et al. 2014). However, less is known about the endocrine mechanisms that regulate body mass and lipid gains during times of gestation and re-feeding (Renouf & Noseworthy 1991; Jeanniard du Dot et al. 2009; Richmond et al. 2010a; Richmond, Norris & Zinn 2010b). Cortisol is a physiological mediator of homeostasis, and as such its effects are diverse (McEwen & Lasley 2002; Goymann & Wingfield 2004). Cortisol secretion typically increases when animals are fasting or in poor condition, and this activates hormone-sensitive lipase and adipose triglyceride lipase that mobilize the body's lipid stores. Increased cortisol also slows the clearance of growth hormone (GH) from circulation. As GH accumulates, lipolysis is promoted in order to conserve lean mass and protein (Ortiz et al. 2003; Richmond et al. 2010a; Richmond et al. 2010b). In contrast, thyroid hormones (TH; triiodothyronine; T_3 and thyroxine; T_4) decrease during fasting to slow metabolic rates through their effects on mitochondrial density and tricarboxylic acid cycle enzyme activities (Yen 2001). On the other hand, when animals are consistently feeding, cortisol production declines (McEwen & Lasley 2002), GH receptors increase in the liver, and GH binding leads to increased insulin-like growth factor (IGF)-1 production (Oster et al. 1995; Rausch et al. 2002; Richmond et al. 2010a). Since IGF-1 plays a major role in depositing lipid and synthesis of lean mass during times of positive energy balance, elevated IGF-1 concentrations generally leads to increases in mass and body condition.

In pinnipeds, the overlap of an energetically-costly annual moult with potentially poor condition such as may be expected at the end of the lactation period, presents the animal with a physiological conflict between hormones associated with hair regeneration and those thought to impact fasting. Both THs and cortisol concentrations rise to facilitate protein incorporation into new fur (Riviere, Engelhardt & Solomon 1977; Ashwell-Erickson, Fay & Elsner 1986; John Ronald & George 1987; Boily 1995; Atkinson, Arnould & Mashburn 2011), and cortisol may serve to trigger a "foraging response" in pinnipeds at the end of the moult (Riviere et al. 1977; Guinet et al. 2004). Yet, increased THs and cortisol both reduce an animal's ability to conserve mass and lipid stores during the moult. In addition, because the moult period is when embryo implantation typically occurs in phocids (Smith 1966; Atkinson 1997), poor body condition and increased cortisol concentrations may negatively impact pregnancy rates (Crocker et al. 2011).

This study aimed to characterize body condition during the post-moult period in Weddell seals and to assess potential hormonal correlates of energy deposition over the winter. In order to assess important aspects of the animal's physiology that are necessary to support gestation, we determined whether the females that successfully produced a pup the following year (t+1) differed in measurable ways from females that skipped reproduction. In light of on-going climatological and ecosystem changes in polar environments, understanding the impact of physiological condition on reproductive success is particularly important.

3.3 Methods

3.3.1 Animal Handling

Adult female Weddell seals were captured on the ice along the McMurdo Sound region, Antarctica in Erebus Bay (~77°S, 165°E) and the Victoria Land coastline (~76°S, 162°E) at the southernmost extent of the species' range. Fifty-three post-moult females were sampled in January/February (austral fall) 2010-2012, and all were assumed to be non-reproductive (did not give birth) that season based on the fact that none of the moulted known-age individuals handled in this study and <15% of fully moulted females surveyed in the population in the fall had a pup a few months prior (October/November; Burns et al. 2013; Beltran & Burns *unpublished*). Forty-

seven females (30 non-reproductive females; 17 reproductive females) were sampled in October/November (austral spring), following the winter foraging period. During the austral spring, reproductive females were handled on average 7.3 ± 1.5 days post-partum, while non-reproductive females were sampled during this same time period, prior to the breeding season (Fig. 3.1). Of the Weddell seals handled in the austral spring, twenty were re-captured animals handled the previous fall (275.5 ± 1.5 days earlier).

Animals were sedated with an initial intramuscular dose of approximately 1.0 $mg \cdot kg^{-1}$ tiletamine/zolazepam HCl (Fort Dodge Laboratories, Inc). Following a 10 to 15 minute induction period, animals were captured via hoop net and additional intravenous injections of ketamine and diazepam (100 $mg \cdot mL^{-1}$; Parnell or Fort Dodge Laboratories and 5 $mg \cdot mL^{-1}$; Parnell or Abbott Laboratories) were administered as necessary to keep animals sedated while remaining eupneic. Animals were weighed using a sling, tripod, and scale (MSI-7200-IT Dyna-Link digital dynamometer, capacity 1,000 ± 1.0 kg) and a standard length (dorsal straight line, nose to tail) measurement was taken.

3.3.2 Body Composition Measurements

Body composition was determined for each animal using the labelled water dilution technique, as described in Shero et al. (2014). Briefly, a pre-injection blood sample was taken, and 1 to 1.5 mCi of tritiated water (HTO) was injected into the extradural vein of each animal. Post-equilibration blood samples were collected ≥90 minutes after injection. Serum was separated from whole blood samples via centrifugation and stored at -80°C prior to analyses. Serum samples were distilled in triplicate, water was extracted using the freeze-capture technique as described in Ortiz, Costa & Le Boeuf (1978), and HTO specific activity (counts per minute; CPM) was determined using a Packard Tri-Carb 2900TR liquid scintillation counter (Packard Bioscience Co., Meriden, CT) to determine total body water (TBW) volume. Samples were distilled again if CVs were >2%. TBW values were reduced by 3.3% to account for post-injection isotope loss (Bowen & Iverson 1998), and percent total body lipid was calculated from TBW following Reilly & Fedak (1990). Lean mass was calculated as the difference between measured total body mass and calculated lipid mass.

3.3.3 Hormone Quantification

At the beginning of the animal handling period, blood samples were taken from the extradural vein in serum separator tube (SST) vacutainers^{TM} (24.0 ± 0.7 min post initial sedation) to assess seasonal differences in cortisol, GH, IGF-1, T_3, and T_4 hormone concentrations. After centrifugation, serum samples were stored at -80°C until analysis. Cortisol and T_3 levels were determined using commercially available human RIA kits (Siemens Healthcare Diagnostics, Coat-a-Count), while T_4 was measured using Siemens' canine assay. GH was measured using a porcine RIA with rabbit anti-porcine antibodies (Barb et al. 1991; Richmond & Zinn 2009), and IGF-1 levels were determined using a bovine RIA with rabbit anti-human IGF-1 antisera as the primary antibody (Johnson et al. 1996; Richmond & Zinn 2009). All assays were validated by parallelism and standard addition tests to confirm no matrix effects existed in Weddell seal serum. Kit standards ran parallel across the range of Weddell seal values and recoveries from pooled serum for all assays were 85-110% (assay sensitivities, cortisol: 0.2 $\mu g \cdot dL^{-1}$, GH: 0.8 $ng \cdot mL^{-1}$, IGF-1: 10 $ng \cdot mL^{-1}$, T_3: 7 $ng \cdot dL^{-1}$, T_4: 0.22 $\mu g \cdot dL^{-1}$). To determine the effect of handling on hormone secretion, variation in concentrations due to time of day and time post sedation were assessed. In addition, variation in cortisol concentrations across the handling period (Champagne et al. 2012b) (time=0, 10, 20, 30, 60, and 90 min following hoop net capture) were measured in a subset of animals. For all RIA assays, CPM was measured on a Genesys Genii series LT1010 (LTI Laboratory Technologies, Inc., Maple Park, IL) or a Perkin Elmer Wallac Wizard 1470 (PerkinElmer, Inc., Waltham, MA) gamma counter alongside kit controls, and samples were re-analysed if CVs of replicates were >10%. Intra- and inter-assay variations were determined for all assays (cortisol: 5.0% and 2.9%, GH: 4.9% and 11.2%, IGF-1: 8.6% and 8.9%, T_3: 13.7% and 9.8%, T_4: 2.7% and 3.4%).

3.3.4 Statistical Analyses

Data were tested for normality prior to statistical analysis, and transformed as necessary. General linear mixed models (GLMMs) with Bonferroni post-hoc comparisons were used to test for differences in physiological status by animal class (fall non-reproductive, spring non-reproductive, spring reproductive) and year. Repeated measures were used to account for the fact that some individuals were handled multiple times (SPSS v. 22, Chicago, IL, USA). Because

year and/or its interactions with animal class were significant factors in many cases, GLMM analyses were run for each study year separately. For model validations, residuals were tested for normality and homoscedasticity, and standardized residuals were used to assess model outliers. To determine whether animals were larger, for their stature, standard length was included as a covariate in statistical models of mass and body composition. Regression analyses were used to assess relationships between mass or body composition with hormone concentrations.

Overwinter changes in physiological parameters of those females handled in both fall and spring were analysed separately using paired t-tests. Additionally, binary logistic regressions were used to assess whether physiological status (total body mass, lipid stores, hormone levels) in fall impacted the likelihood of having a pup the following spring, and whether physiological parameters differed within spring for individuals with a pup versus non-reproductive animals. Significance was defined as $\alpha = 0.05$ throughout, and results are reported as mean ± SE.

3.4 Results

3.4.1 Body Size & Composition

Overall, standard length did not vary by season in any study year, suggesting that animals were of similar age in both seasons; however, reproductive females in spring were significantly longer than non-reproductive animals. These differences were only significant in years 2010 and 2011. In 2012, standard length did not differ among animal classes (Table 3.1). As expected, standard length accounted for a significant amount of the variation in total body mass in all study years with longer animals being significantly heavier (2010: $F_{1,29.7}$=46.3, P<0.001; 2011: $F_{1,20.0}$=64.4, P<0.001; 2012: $F_{1,12.1}$=19.9, P=0.001).

After controlling for animal length, total body mass and lipid stores tended to be larger in spring following the winter foraging period. Total body mass differed by reproductive group in both 2010 and 2012, but not 2011 (Table 3.1). All seals handled in spring 2010 were significantly heavier than post-moult non-reproductive females the previous fall. In 2012, non-reproductive females in spring weighed significantly more than both reproductive females and post-moult animals from the fall. Seasonal and annual differences in condition did not always track changes

in mass. For example, overwinter increases in lipid stores were significant in 2011 and 2012 (Table 3.1). Both non-reproductive and reproductive seals in spring were in significantly better condition following the winter foraging period than females handled in fall. However, these seasonal trends were not significant in the 2010 study year. Within the spring pre-breeding period, the body composition of females that gave birth and those that skipped reproduction was always similar.

Animals that were first weighed as non-reproductive females in fall and then again following the long winter foraging period were significantly larger in spring, regardless of their reproductive status the following year (Fig. 3.2; reproductive: t_{10}=-2.8, P=0.018, Δ+10.6 ± 3.8% total body mass, range: Δ-6.7 to +29.2%; non-reproductive: t_4=-3.0, P=0.038; Δ+15.5 ± 5.5% total body mass, range: Δ-0.5 to +29.8%). Females in both reproductive categories also gained significant amounts of lipid mass over the winter foraging period (reproductive: t_8=-5.7, P<0.001; non-reproductive: t_4=-7.3, P=0.002), but there was no increase in lean body mass (reproductive: t_8=-0.7, P=0.476; non-reproductive: t_4=-1.6, P=0.187). The only difference in the pattern of overwinter gains between reproductive groups, was that females that had a pup in year t+1 showed a significant increase in lipid as a proportion of body mass, while the non-reproductive females did not (reproductive: t_8=-6.4, P<0.001, non-reproductive: t_4=-2.2, P=0.088).

3.4.2 Endocrinology

Neither time of day, nor the amount of time between initial sedation and animal restraint, were significant factors in GLMM models (i.e., cortisol, Fig. 3.3A $F_{11,61.1}$=1.2, P=0.334), and were subsequently removed from analyses of hormone concentrations. Specifically looking at cortisol, there were no significant differences in cortisol concentrations in fall (Fig. 3.3B; $F_{5,2.9}$=6.5, P=0.081) across the entire animal handling procedure, and levels were only elevated at 90 mins post-physical restraint in spring (Fig. 3.3B; non-reproductive: $F_{5,14.6}$=6.2, P=0.003; reproductive: $F_{5,12.3}$=5.0, P=0.010). Therefore, hormone concentrations measured from initial blood samples were used for seasonal comparisons.

In the cross-sectional study, reproductive females tended to have lower cortisol levels than non-reproductive seals; however, this trend was only significant in the 2011 study year (Table 3.2). Circulating GH concentrations were slightly or significantly higher in animals handled in fall, as

compared to spring. However, differences in GH between reproductive and non-reproductive females varied on a year-to-year basis. In 2010, post-moult females in the fall had significantly higher circulating GH than either reproductive or non-reproductive females in spring. But in 2012, females handled in fall and reproductive females handled in spring had higher GH concentrations than spring non-reproductive females. IGF-1 differed by reproductive class in 2010 and 2011. In 2010, IGF-1 was significantly higher in spring non-reproductive females than in fall. However, in 2011, spring non-reproductive females had significantly lower IGF-1 concentrations than either non-reproductive seals in the fall or reproductive females in spring. There were no differences in IGF-1 concentrations among animal groups in 2012.

T_3 did not vary due to season or reproductive category in any study year (Table 3.2). On the other hand, T_4 differed among animal classes in all study years, although the pattern was not consistent. In 2010 and 2011, T_4 was significantly higher during the fall post-moult period as compared to both reproductive groups in spring. But in 2012, the overwinter decline in T_4 was only apparent in the reproductive females. These seasonal and/or reproductive differences in T_4 altered T_3:T_4 ratios, so that the ratio tended to be higher in spring following the winter foraging period. Increases in the T_3:T_4 ratio overwinter were significant in 2010 regardless of reproductive status in the spring, and in 2011 the T_3:T_4 ratio was significantly higher in non-reproductive females in the fall as compared to the spring.

The same seasonal trends in endocrine factors seen at the population level were present in the longitudinal study. Females that returned the following year and skipped reproduction exhibited a slight, but non-significant, increase in cortisol concentrations (Fig. 3.4A; t_7=-1.7, P=0.125), and females that returned and gave birth showed a slight decrease (t_{11}=1.5, P=0.154). In contrast, GH levels decreased over the winter in non-reproductive females (Fig. 3.4B; t_5=3.6, P=0.015), but not in reproductive seals (t_8=0.5, P=0.662). There were no seasonal changes in IGF-1 concentrations for females that returned with a pup the following year (Fig. 3.4C; t_{11}=0.1, P=0.947), or not (t_7=0.4, P=0.714). Similarly, there were no seasonal differences in T_3 concentrations in any reproductive class (Fig. 3.4D, non-reproductive: t_7=1.0, P=0.342; reproductive: t_{11}=-0.1, P=0.938). In contrast, T_4 was significantly lower in spring for both non-reproductive (Fig. 3.4E; t_7=3.5, P=0.010) and reproductive females (t_{11}=4.6, P=0.001). Females that returned without a pup showed no significant seasonal difference in their T_3:T_4 ratios (Fig.

3.4F; t_7=-0.1, P=0.944), while females that gave birth had significantly higher T_3:T_4 ratios in spring (t_{11}=-2.5, P=0.030).

3.4.3 Linking Body Condition, Hormones, and Reproduction

There were significant relationships between mass and body composition with measured hormone concentrations, but patterns were not consistent across reproductive groups. For example, GH was negatively correlated with body mass during the post-moult period (Fig. 3.5A, $F_{1,49}$=19.6, P<0.001, GH=-0.125[Mass]+64.643, R^2=0.290), and in spring non-reproductive females ($F_{1,26}$=10.0, P=0.004, GH=-0.056[Mass]+28.574, R^2=0.286), but not in spring reproductive females. IGF-1 concentrations were positively correlated with body mass in reproductive females (Fig. 3.5B, $F_{1,14}$=7.3, P=0.018, IGF-1=0.466[Mass]–121.129, R^2=0.359), but not the non-reproductive females in either season. T_3 was negatively correlated with mass only in spring reproductive females (Fig. 3.5C, $F_{1,15}$=4.9, T_3=-0.196[Mass]+152.434, R^2=0.260). Conversely, T_4 exhibited a positive relationship with body mass, but only in non-reproductive females in spring (Fig. 3.5D, $F_{1,27}$=4.8, P=0.037, T_4=0.002[Mass]+1.039, R^2=0.157) and none of the other animal groups. There was also a positive correlation between T_3 and T_4 concentrations with body composition for reproductive animals handled in spring (Fig. 3.5E-F, T_3- $F_{1,14}$=8.1, P=0.014, T_3=2.942[%Lipid] – 38.850, R^2=0.383; T_4- $F_{1,14}$=4.7, P=0.049, T_4=0.054[%Lipid]–0.307, R^2=0.265), but this pattern was not present in non-reproductive females.

In the longitudinal study, the probability that a female handled in fall would return with a pup the subsequent spring was greater if she had higher T_4 levels during the previous fall (Fig. 3.6A; χ^2=6.3, P=0.012), lower cortisol concentrations in spring (Fig. 3.6B; χ^2=4.0, P=0.046), or if GH concentrations were maintained at higher levels across the winter (Fig. 3.6C; χ^2=8.3, P=0.004). Mass, body composition, and the other measured hormones did not impact the likelihood of having a pup.

3.5 Discussion

This study showed that the overwinter foraging period is important for female Weddell seals to gain body mass and condition (lipid stores). Yet, despite expectations that reproductive females

would gain more mass than females that returned without a pup, there were no differences in body composition between reproductive groups, either in the cross-sectional or longitudinal study.

During the 8-month overwinter foraging period, Weddell seals must fuel the costs of maintenance metabolism and their daily activities (average daily metabolic rate, ADMR; Kleiber 1975; Lavigne et al. 1982) and store excess energy as lean and lipid tissue (Table 3.3). Because post-partum and non-reproductive females gained similar amounts of body mass over this period, the only differences in net energy demands appear to be resources allocated towards foetal and placental tissue accretion (Lavigne & Stewart 1979; Wheatley et al. 2006) and the heat increment of gestation (HIG; Brody 1945). If total body mass is extrapolated to parturition date for our females using post-partum rates of mass loss from Wheatley et al. (2006), this study suggests that pregnant Weddell seals gain 25.9% body mass over the austral winter. However, only 17.2% of this body mass gain (62.5 kg or 1691.5 MJ) is allocated to the female for self-maintenance, with the rest consumed by pregnancy costs when considering the addition of the 25-30 kg pup (Table 3.3; Wheatley et al. 2006). Based on changes in body mass and composition, Weddell seal lactation costs are 4286 MJ, but this does not account for additional resources females may acquire while foraging (Wheatley et al. 2006). Thus, at a maximum, female Weddell seals only gain 39.5% of the energy required for the nursing period across the winter, clearly indicating why females cannot rely entirely on a capital breeding strategy. In contrast, other phocid seals such as grey seals (*Halichoerus grypus*) and northern elephant seals (*Mirounga angustirostris*) gain >60-100% of their body mass during their at-sea gestation period (Table 3.3; Beck et al. 2003; Robinson et al. 2012). In these species, a greater proportion of energetic gain is devoted to self-preparation during gestation (i.e., more energy intake is laid down as tissue) than Weddell seals. Also in contrast to Weddell seals, grey seals and northern elephant seals gain 59 and 89% of the energy needed to fuel lactation, respectively (Table 3.3; Costa et al. 1986; Anderson & Fedak 1987; Beck et al. 2003; Robinson et al. 2012).

Based on these comparisons, female Weddell seals do not gain as much mass or energy over their eight-month long foraging period as other phocid species. Low light and heavy ice conditions across the austral winter likely reduce prey profitability and make it difficult for this species to recuperate mass. Therefore, while it is well-established that the lactation period is the

most energetically-costly part of reproduction in pinnipeds (Fedak & Anderson 1982; Lavigne et al. 1982; Costa et al. 1986), accumulating energy reserves during gestation may be a substantial obstacle in bringing a foetus to term for Weddell seals. Insufficient overwinter recuperation may explain findings by Hadley, Rotella & Garrott (2007) that reproduction decreases yearly Weddell seal survivorship probabilities by 3%, and that Weddell seals have high rates of skip-breeding (1 out of 3 years; Testa 1987; Hadley, Rotella & Garrott 2006) as compared with other phocid species. If so, then supplementing energy reserves by summer foraging during or after lactation may be critically important to prevent females from dropping below a threshold body condition that would impact future survival (Crocker et al. 2001). This study adds to a list of others suggesting that a pure capital breeding strategy is not as common in phocid seals as previously thought (Kelly & Wartzok 1996; Boyd 1998; Haulena, St. Aubin & Duignan 1998; Lydersen & Kovacs 1999; Bowen et al. 2001).

Because Weddell seals forage throughout late summer and the entire austral winter to accumulate a relatively small proportion of the energy reserves needed to support reproductive success, the allocation of energy is likely tightly regulated by intrinsic factors. When post-moult animals were at their leanest in the fall, cortisol levels were not elevated, suggesting that losing mass and lipid stores during this physiologically "programmed" period does not increase allostatic load (Romero, Dickens & Cyr 2009). Alternatively, Weddell seals are known to have the highest cortisol concentrations of any mammal (Liggins et al. 1993), and any seasonal variations may be in cortisol bioavailability and how much hormone is tied-up by corticosteroid-binding globulin (Breuner & Orchinik 2002). Decreases in cortisol concentrations in other species during gestation have been shown to be beneficial to foetal growth (Challis et al. 2001; Jensen et al. 2002), and may function similarly in Weddell seals. It is not surprising that GH was elevated and IGF-1 concentrations were low in females that had just completed the moult. Because these animals were coming to the end of a period of reduced feeding, higher GH concentrations would promote lipid oxidation and defend lean mass, while low IGF-1 would indicate animals were not accreting new lean mass (Ortiz et al. 2003).

As expected due to its role in hair regeneration (Ramot et al. 2009), T_4 was slightly or significantly higher during the post-moult period but the biologically-active T_3 form did not vary by season. This led to lower T_3:T_4 ratios in fall, which may be due to a larger fraction of T_4 being

deiodinated to the inactive reverse T_3 (rT_3) form (Haulena et al. 1998), or to faster TH flux by deiodination and binding of the biologically-active T_3 form at target tissues, which would elevate T_4 (Weingartner et al. 2012). These two situations would produce different changes in metabolic rate; however, this study is not able to address which mechanism is occurring in post-moult Weddell seals. Increased T_4 and GH concentrations have also been associated with the onset of foraging in other phocids and terrestrial mammals (Boness, Bowen & Oftedal 1994; Escobar-Morreale, Escobar del Rey & Morreale de Escobar 1997; Haulena et al. 1998; Amin, Dhillo & Murphy 2011; Florant & Healy 2012), and may play a role in the initiation of winter foraging in the Weddell seal. Across the austral winter, GH decreased while animals gained mass and condition, but this pattern was less apparent in reproductive animals. Keeping higher GH concentrations across the winter in reproductive seals may lead to less protein utilization while supporting the additional costs of gestation and subsequent costs of lactation. GH turnover and receptors in the liver should increase when animals are well-fed, facilitating production of IGF-1. Yet, relative to other pinnipeds (Richmond et al. 2010a; Richmond et al. 2010b; Crocker et al. 2012), Weddell seals had fairly low IGF-1 concentrations in both study seasons suggesting that animals may defend lean tissue mass throughout the year.

Integrating changes in body condition and hormone values allows us to make broader conclusions about the importance of overwinter foraging in Weddell seals. During the post-moult period, neither mass nor condition was predictive of future pupping in year t+1. Only those females with higher circulating T_4 concentrations in fall were significantly more likely to have a pup the following year, and adequate TH levels increase the probability of conception and lower chances of spontaneous abortion in humans and other species (Abalovich et al. 2002; Vissenberg et al. 2012). In humans, THs play a major role in ovulation, mediating embryo implantation, and the maintenance of early pregnancy and foetal development (Fitko et al. 1996; Ashkar et al. 2010; Dittrich et al. 2011; Colicchia et al. 2014). TH receptors on both the female's uterine endometrium and the blastocyst increase during the window of embryo attachment, and TH binding increases synthesis of integrins and enzymes that directly influence the implantation process (Oki et al. 2004; Colicchia et al. 2014). After embryo attachment, maternal THs decrease apoptosis of blastocyst cells, enhance foetal cell division and growth rates, and provide the foetus with TH until its thyroid gland develops (Contempré et al. 1993; Ashkar et al. 2010). Therefore,

in Weddell seals, elevated T_4 at the end of the moult may not only serve as a metabolic regulator and facilitator of hair regrowth, but may also promote positive reproductive outcomes.

The endocrine profiles of non-reproductive animals suggest they need to conserve total body mass and lipid stores, while post-partum female profiles are tailored to prioritize transfer of energy reserves to the pup. In spring, larger reproductive females had higher IGF-1 concentrations, suggesting that they were allocating excess energy to both lipid and lean body mass deposition over the winter. Post-moult females in fall and smaller non-reproductive females in spring had higher GH concentrations, likely conserving lean mass. Yet, this relationship did not exist in lactating females, possibly because some protein must be catabolized to supply the pup with nutritionally-complete milk (Costa et al. 1986). Smaller non-reproductive females also tended to have higher T_4 levels in spring, suggesting that while these animals are conserving lean tissue mass, they are still utilizing lipid. Indeed, post-partum females with greater lipid stores had higher T_3 and T_4 levels, which would facilitate greater mobilization of lipid to the milk, rather than for maternal maintenance. It would be advantageous for larger females to allocate a greater proportion of energy reserves to their pup, to increase first year survival rates (Proffitt, Garrott & Rotella 2008).

In summary, Weddell seals gain less mass and condition during gestation than other phocid seals. Females do not accumulate enough stores over the winter to support lactation, a potential reason for the intermediate income-capital breeding strategy. The fact that Weddell seals are apparently unable to gain substantial amounts of mass over the winter may increase their vulnerability to changes in ecosystem structure, such as may occur in response to the Ross Sea toothfish fishery (*Dissostichus mawsoni*; DeVries, Ainley & Ballard 2008; Ainley & Siniff 2009; Ainley et al. 2012). That there was annual variation in physiological condition suggests some flexibility to cope with environmental changes such as sea-ice extent and large-scale oceanographic El-Niño Southern Oscillation events that influence primary productivity (Testa 1994; Wheatley et al. 2006; Hadley et al. 2006; Hadley et al. 2007; Proffitt et al. 2007; 2008; Chambert, Rotella & Garrott 2012; Chambert et al. 2013). Patterns of modest energetic gains during gestation and interannual variation in physiological parameters may be magnified in this particular study, since the Erebus Bay Weddell seals are at the southernmost extent of this species' range, and seasonality in McMurdo Sound is more extreme than lower-latitude populations would endure.

3.6 Acknowledgements

We thank field team members: Linnea Pearson, Kimberly Goetz, Dr. Patrick Robinson, Dr. Luis Hückstädt, and Dr. Michelle LaRue for sample collection, and also group B-009-M led by Drs. Robert Garrott, Jay Rotella, and Thierry Chambert for their help locating study animals, and Dr. C. Loren Buck, Melanie Richter, and Danielle Dillon for assistance with lab analyses. We are also grateful to Drs. Carrie Eischens and Daniel Crocker for providing data for the species energetics comparison, and two anonymous reviewers whose suggestions improved this manuscript. Logistical support was provided by the National Science Foundation (NSF) U.S. Antarctic Program, Raytheon Polar Services, and Lockheed Martin ASC; we thank all the support staff in Christchurch, NZ and McMurdo Station. This research was conducted with support from NSF ANT-0838892 to D.P.C. and ANT-0838937 to J.M.B. This material is based upon work supported by the National Science Foundation Graduate Research Fellowship under Grant No. DGE-1242789. Any opinion, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation. M.R.S. and this research were supported in part by a UAF Center for Global Change Student Research Grant with funds from the Cooperative Institute for Alaska Research and the Alaska Climate Science Center. Animal handling protocols were approved by the University of Alaska Anchorage and University of California Santa Cruz's Institutional Animal Care and Use Committees. Research and sample import to the United States was authorized under the Marine Mammal permit No. 87-1851-04 issued by the Office of Protected Resources, National Marine Fisheries Service. Research activities were approved through Antarctic Conservation Act permits while at McMurdo Station.

3.7 Data Accessibility

Data used in this manuscript are archived at the United States Antarctic Program Data Center, with files provided through the permanent link http://gcmd.nasa.gov/getdif.htm?NSF-ANT08-38892 (Shero et al. 2015).

Figure 3.1. An adult female Weddell seal (*Leptonychotes weddellii*) just after the annual moult in January/February, with a new silver pelage (*above*; photo courtesy of J. Burns), and a post-partum female during the lactation period. Reproductive and non-reproductive females were handled following the winter foraging period (October/November; *below*, photo by M. Shero), prior to the breeding season and mating (December).

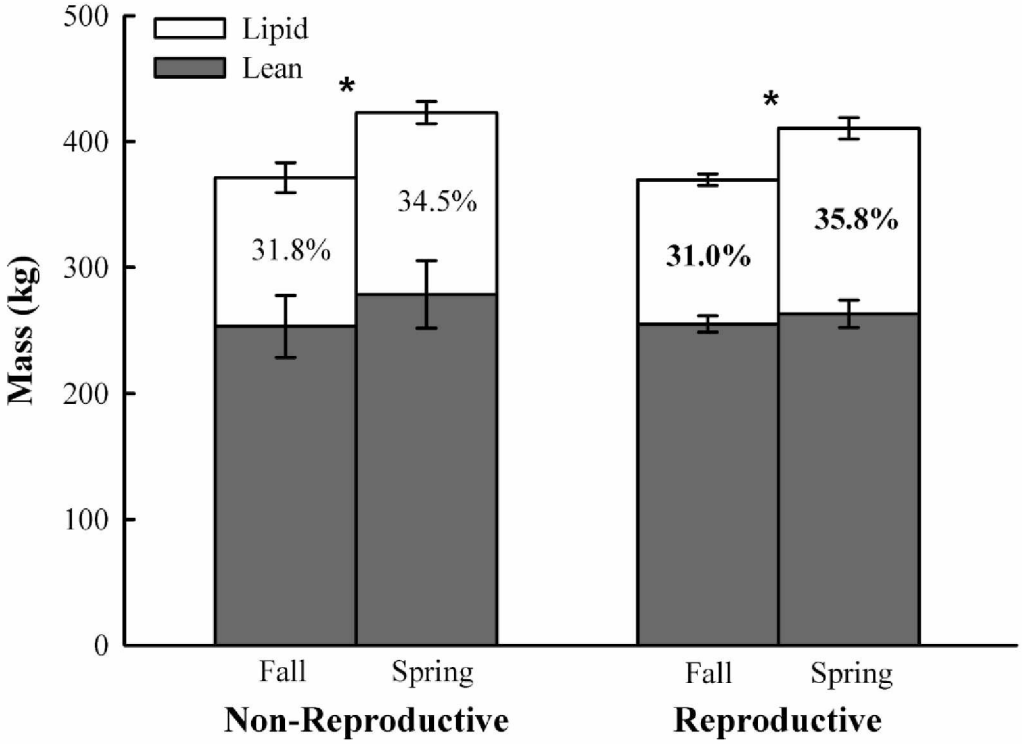

Figure 3.2. Mean ± SE overwinter changes in female Weddell seal body mass and condition. Females that returned in year t+1 and skipped reproduction (*N*= 5) and females that gave birth (*N*= 9) all gained mass and condition (*Asterisk*=significant gains in total body and lipid mass). Percentages show the proportion total body mass comprised of lipid (*Bold*=significant overwinter increase in %lipid). There were no significant differences between values from reproductive and non-reproductive seals within either the fall or spring seasons.

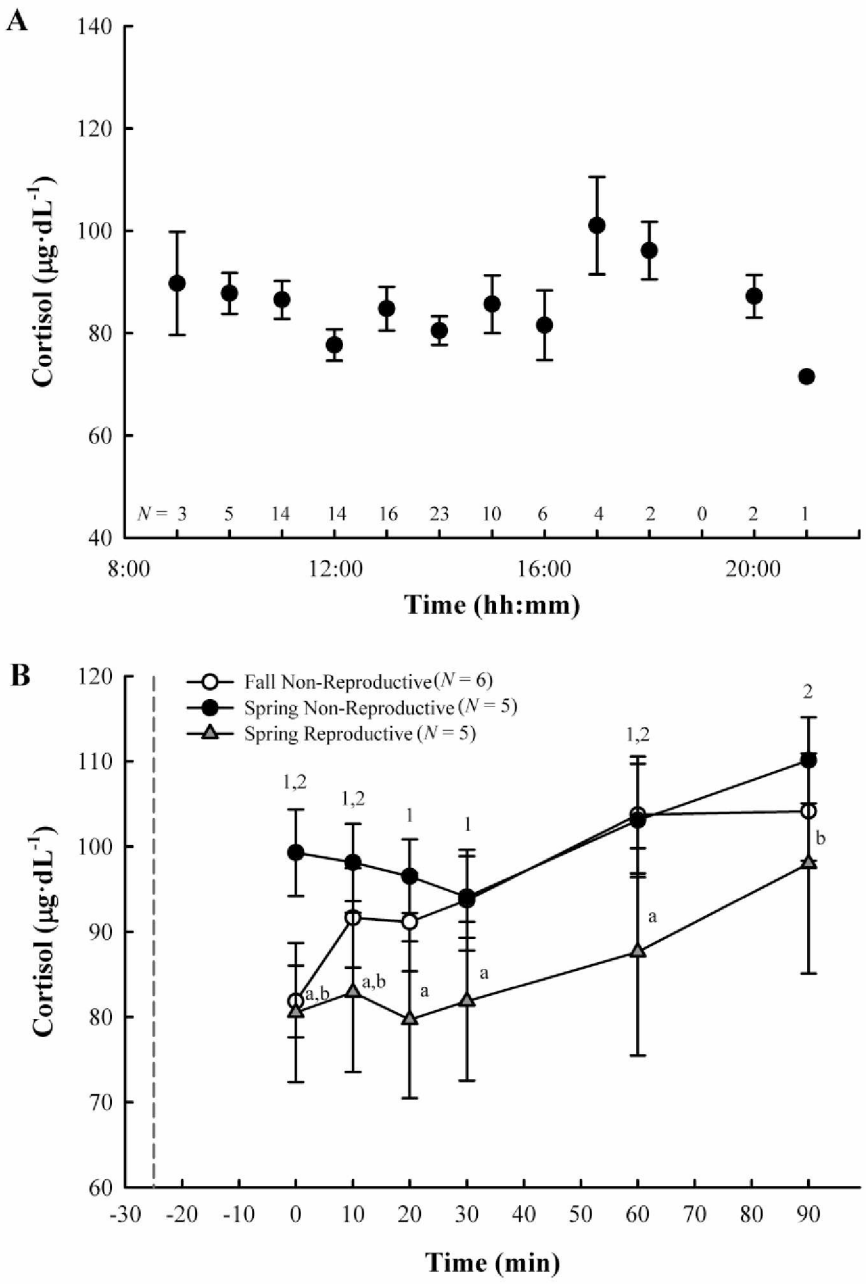

Figure 3.3. (**A**) Mean ± SE serum cortisol concentrations (µg·dL^{-1}) across the day and (**B**) across the animal handling period. Dashed line shows time from initial sedation to physical restraint at time zero. Significant differences in cortisol concentrations between sampling time points are denoted with different numbers and letters, for spring non-reproductive and reproductive seals, respectively.

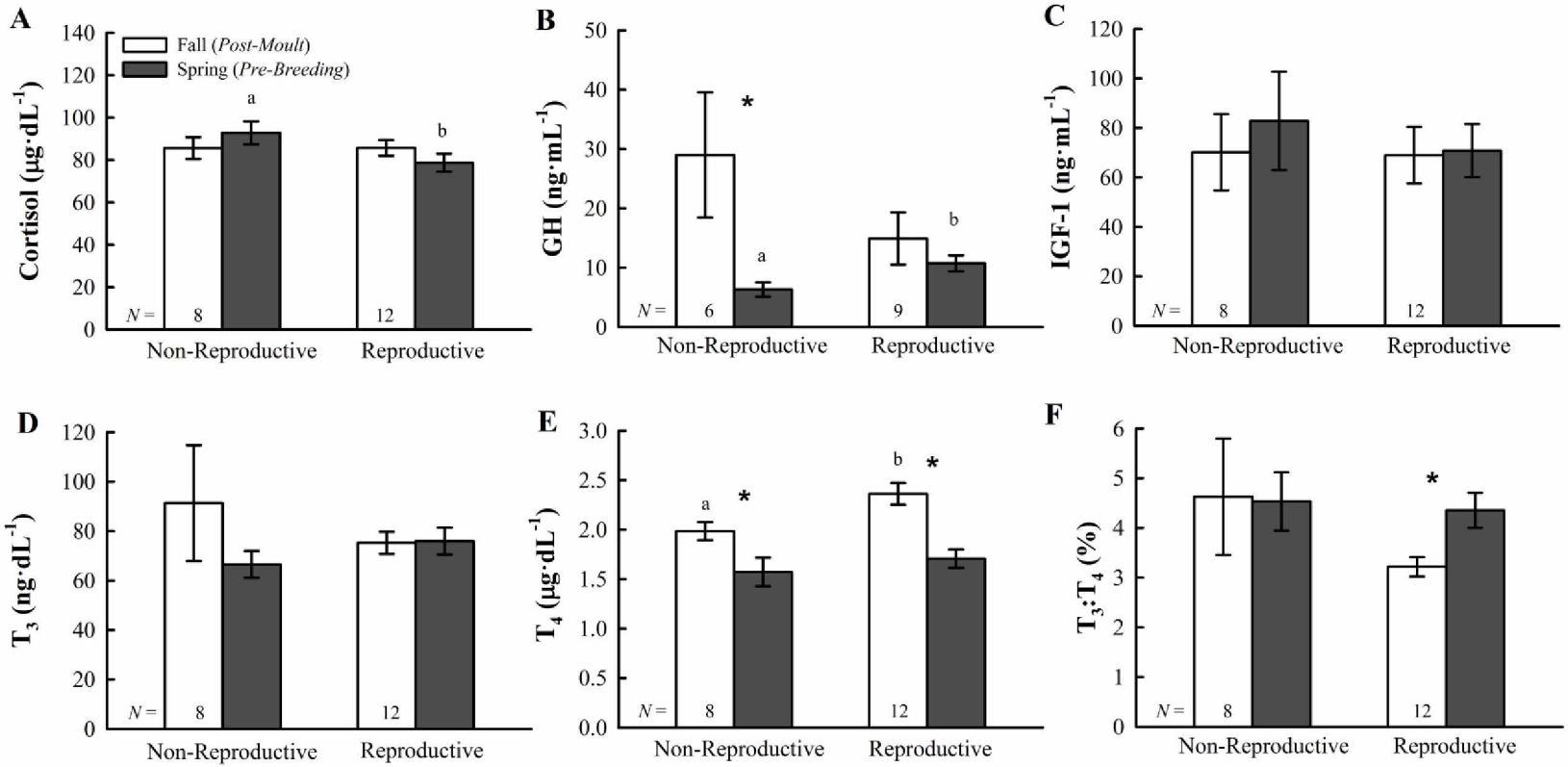

Figure 3.4. Mean ± SE overwinter changes in stress hormone, cortisol (**A**) levels, and metabolic hormones (**B-F**) for female Weddell seals that returned the following year (t+1) and skipped reproduction, or that gave birth in year t+1. *Asterisk*=significant seasonal differences within a reproductive group. *Different letters*=significant differences between reproductive groups within a given season.

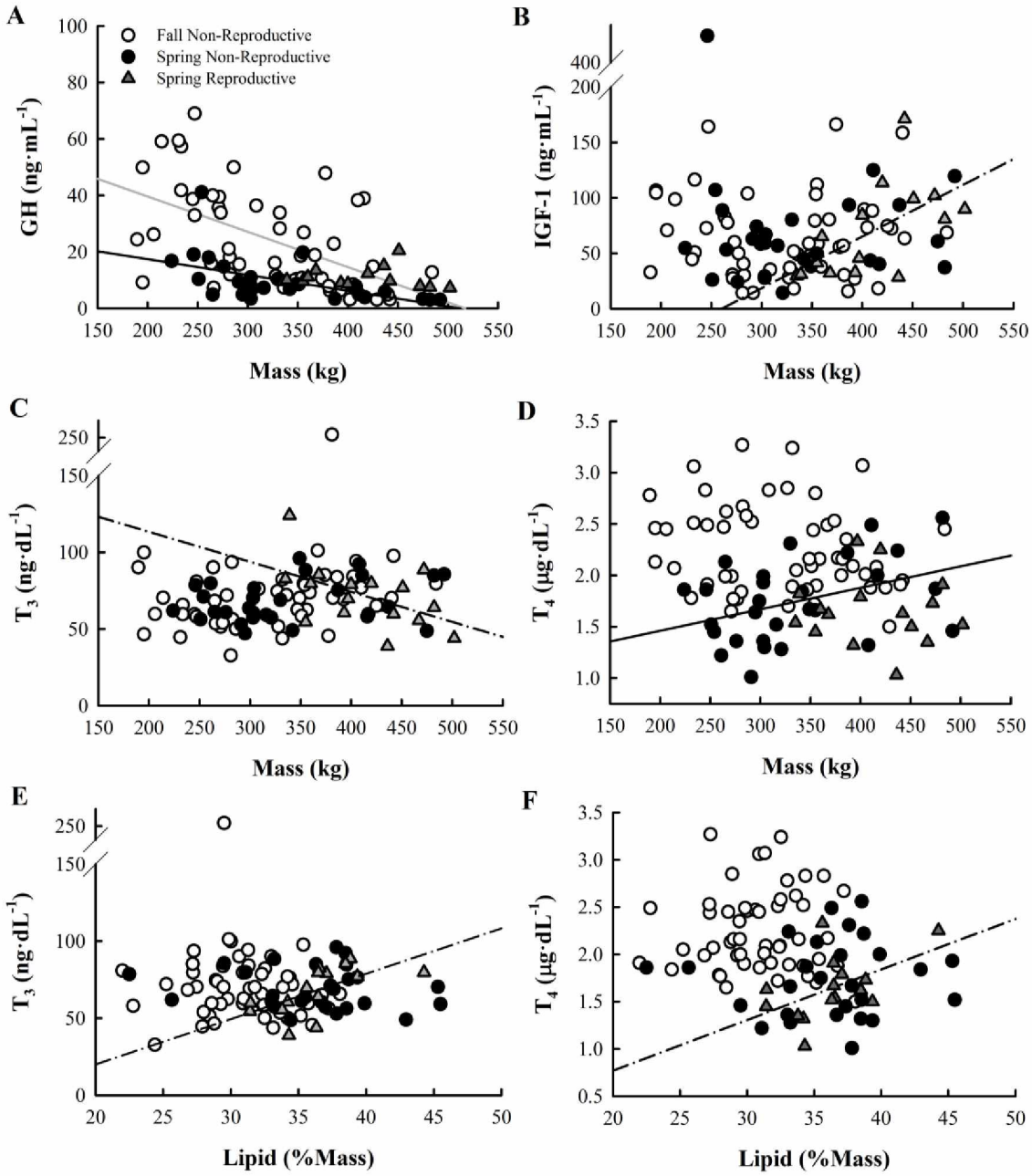

Figure 3.5. Regressions between metabolic hormones and body mass (**A-D**) or body composition (**E-F**) in Weddell seals during the post-moult period in fall and pre-breeding period in spring. Solid grey or black line shows significant relationship in fall or spring non-reproductive female groups, respectively. Dashed line shows a significant relationship between hormone levels and body composition in reproductive females in spring.

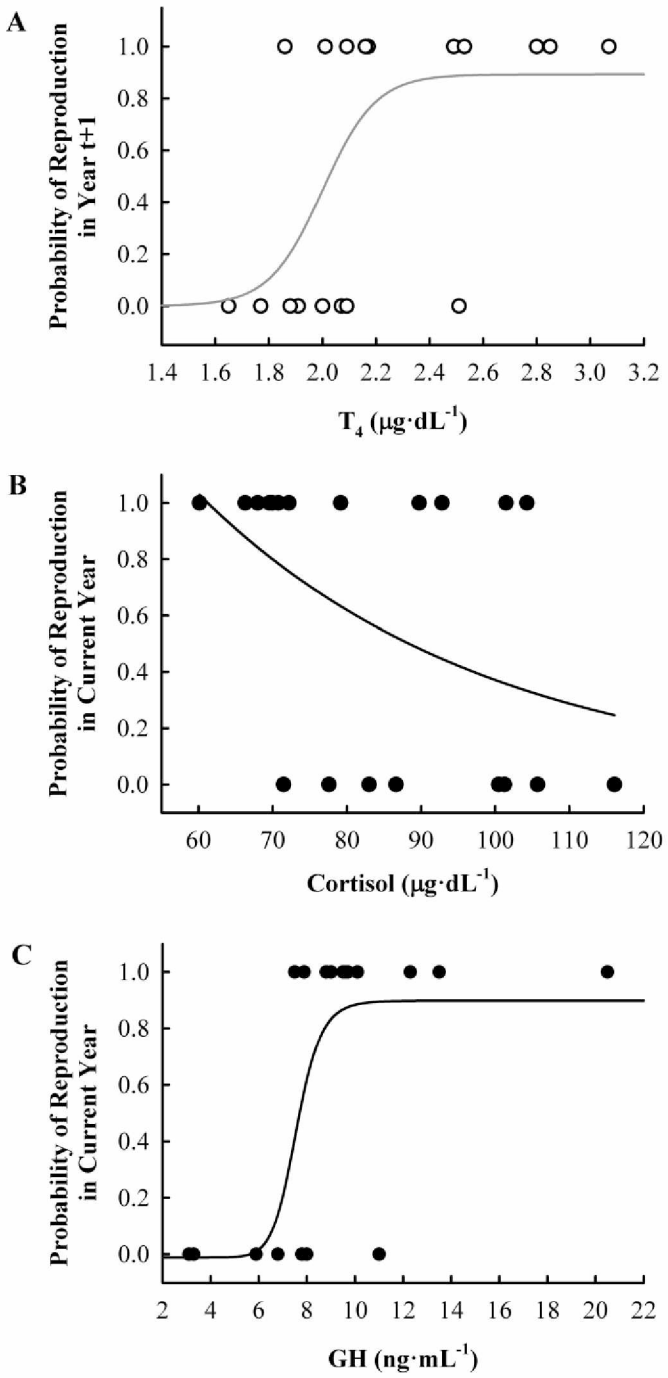

Figure 3.6. (**A**) Serum T_4 concentrations of Weddell seals during the post-moult period in fall influenced the probability of having a pup the following year (t+1). Post-partum females in spring also had lower cortisol levels (**B**) and higher GH concentrations (**C**).

Table 3.1. Mean ± SE female Weddell seal standard length, mass, and body composition (%lipid) for study years 2010-2012. Sample sizes are shown in parentheses and *different letters* indicate significant differences in physiological parameter between animal classes.

	Fall (Post-moult)	*Spring (Pre-breeding)*		
	Non-Reproductive	Non-Reproductive	Reproductive	F ratio, P value (Mix Model)
		2010		
Standard Length (cm)	227.2 ± 3.4 (*18*)[a]	221.9 ± 5.8 (*12*)[a]	252.2 ± 3.4 (*5*)[b]	$F_{2,19.1}$=5.6, P=0.014
Mass (kg)	274.9 ± 13.9 (*18*)[a]	301.8 ± 21.7 (*11*)[b]	433.6 ± 16.4 (*5*)[b]	$F_{2,19.1}$=12.4, P<0.001
Lipid (%Mass)	31.3 ± 0.7 (*18*)	32.2 ± 1.6 (*10*)	35.6 ± 1.0 (*5*)	$F_{1,12.3}$=2.3, P=0.141
		2011		
Standard Length (cm)	228.7 ± 4.0 (*19*)[a]	231.3 ± 3.4 (*12*)[a,b]	253.0 ± 5.8 (*6*)[b]	$F_{2,19.4}$=5.1, P=0.017
Mass (kg)	312.8 ± 15.2 (*19*)	316.1 ± 13.9 (*11*)	427.2 ± 30.1 (*5*)	$F_{2,7.3}$=0.9, P=0.452
Lipid (%Mass)	29.4 ± 1.0 (*18*)[a]	39.3 ± 1.1 (*11*)[b]	37.8 ± 1.6 (*5*)[b]	$F_{2,21.5}$=21.8, P<0.001
		2012		
Standard Length (cm)	248.3 ± 3.0 (*16*)	242.7 ± 7.1 (*6*)	244.2 ± 3.8 (*6*)	$F_{2,7.7}$=0.1, P=0.943
Mass (kg)	384.8 ± 12.7 (*16*)[a]	433.3 ± 15.7 (*6*)[b]	385.8 ± 19.6 (*6*)[a]	$F_{2,22.0}$=6.7, P=0.005
Lipid (%Mass)	32.0 ± 0.7 (*16*)[a]	36.6 ± 1.0 (*6*)[b]	35.4 ± 1.7 (*5*)[b]	$F_{2,9.0}$=14.6, P=0.002

Table 3.2. Mean ± SE Weddell seal hormone concentrations for study years 2010-2012. Sample sizes are shown in parentheses and *different letters* indicate significant difference in hormone levels between animal classes.

	Fall (Post-moult)	*Spring (Pre-breeding)*		
	Non-Reproductive	Non-Reproductive	Reproductive	F ratio, P value (Mix Model)
		2010		
Cortisol ($\mu g \cdot dL^{-1}$)	83.4 ± 3.4 (*18*)	95.5 ± 6.5 (*13*)	81.1 ± 5.9 (*5*)	$F_{2,5.3}$=2.6, P=0.165
GH ($ng \cdot mL^{-1}$)	34.6 ± 4.0 (*18*)^{a}	11.1 ± 1.8 (*12*)^{b}	10.2 ± 1.7 (*4*)^{b}	$F_{2,7.1}$=29.3, P<0.001
IGF-1 ($ng \cdot mL^{-1}$)	53.7 ± 7.1 (*18*)^{a}	105.3 ± 31.4 (*13*)^{b}	61.9 ± 18.4 (*4*)^{ab}	$F_{2,11.4}$=5.9, P=0.018
T_3 ($ng \cdot dL^{-1}$)	66.3 ± 3.7 (*18*)	68.6 ± 3.4 (*13*)	64.5 ± 8.8 (*5*)	$F_{2,28.0}$=0.1, P=0.895
T_4 ($\mu g \cdot dL^{-1}$)	2.37 ± 0.10 (*18*)^{a}	1.60 ± 0.09 (*13*)^{b}	1.44 ± 0.14 (*5*)^{b}	$F_{2,20.5}$=28.1, P<0.001
T_3:T_4 (%)	2.84 ± 0.15 (*18*)^{a}	4.49 ± 0.37 (*13*)^{b}	4.40 ± 0.23 (*5*)^{b}	$F_{2,24.0}$=16.4, P<0.001
		2011		
Cortisol ($\mu g \cdot dL^{-1}$)	83.3 ± 3.5 (*19*)^{a}	82.4 ± 3.6 (*12*)^{a}	74.9 ± 3.3 (*6*)^{b}	$F_{2,8.5}$=12.2, P=0.003
GH ($ng \cdot mL^{-1}$)	20.7 ± 4.5 (*16*)	10.7 ± 3.1 (*11*)	9.1 ± 0.9 (*5*)	$F_{2,9.4}$=4.0, P=0.057
IGF-1 ($ng \cdot mL^{-1}$)	69.8 ± 8.6 (*18*)^{a}	45.6 ± 7.8 (*12*)^{b}	71.6 ± 12.4 (*6*)^{a}	$F_{2,8.5}$=6.5, P=0.020
T_3 ($ng \cdot dL^{-1}$)	70.7 ± 4.3 (*19*)	63.0 ± 3.6 (*12*)	67.6 ± 5.6 (*6*)	$F_{2,16.3}$=1.0, P=0.388
T_4 ($\mu g \cdot dL^{-1}$)	2.38 ± 0.11 (*19*)^{a}	1.59 ± 0.11 (*12*)^{b}	1.82 ± 0.17 (*6*)^{b}	$F_{2,23.2}$=14.7, P<0.001
T_3:T_4 (%)	3.04 ± 0.20 (*19*)^{a}	4.11 ± 0.28 (*12*)^{b}	3.81 ± 0.41 (*6*)^{ab}	$F_{2,15.3}$=7.0, P=0.007
		2012		
Cortisol ($\mu g \cdot dL^{-1}$)	83.6 ± 3.2 (*16*)	93.4 ± 7.2 (*6*)	80.1 ± 6.7 (*6*)	$F_{2,16.2}$=1.3, P=0.288
GH ($ng \cdot mL^{-1}$)	16.9 ± 3.1 (*16*)^{a}	4.8 ± 0.8 (*6*)^{b}	12.4 ± 1.7 (*6*)^{a}	$F_{2,9.5}$=13.3, P=0.002
IGF-1 ($ng \cdot mL^{-1}$)	70.5 ± 10.9 (*16*)	75.6 ± 13.9 (*6*)	73.3 ± 22.2 (*6*)	$F_{2,21.0}$=0.3, P=0.774
T_3 ($ng \cdot dL^{-1}$)	81.4 ± 12.0 (*16*)	75.1 ± 6.6 (*6*)	80.1 ± 10.1 (*6*)	$F_{2,23.3}$=0.1, P=0.989
T_4 ($\mu g \cdot dL^{-1}$)	2.01 ± 0.07 (*16*)^{a}	2.12 ± 0.19 (*6*)^{a}	1.62 ± 0.05 (*6*)^{b}	$F_{2,19.7}$=4.8, P=0.020
T_3:T_4 (%)	4.09 ± 0.60 (*16*)	3.76 ± 0.66 (*6*)	4.90 ± 0.47 (*6*)	$F_{2,23.2}$=0.6, P=0.561

Table 3.3. Mean ± SE overwinter mass changes and energy demand for Weddell seals that skipped reproduction the following year, and those that produced a pup. Energetic demand is considered the amount of energy in accreted tissues and metabolic costs of maintenance and/or gestation. The differences in gains between non-reproductive and reproductive seals were taken to be the cost of producing the pup. Comparisons of energy accumulation across gestation among other phocid species are also included.

Species	Source	Time between handlings (days)	Parameter	Mass Gain (kg)	Mass Gain (%)	Mass Gain kg·day^{-1}	MJ Demand	MJ·day^{-1}
Weddell seal								
Non-Reproductive	*This Study*	274.6 ± 1.9	Female body condition gains[1]	51.6 ± 17.0	15.5 ± 5.5	0.19 ± 0.06	1,141.2 ± 186.5	4.15 ± 0.67
N = 5			Average Daily Metabolic Rate (ADMR)[2]	---	---	---	14,264.7 ± 903.9	52.0 ± 3.4
			Summed: female body composition change, with demand of ADMR	51.6 ± 17.0	15.5 ± 5.5	0.19 ± 0.06	15,406.0 ± 823.9	56.0 ± 3.14
Reproductive	*This Study*	274.2 ± 2.1	Female body condition gains, ~7 days post-partum	38.7 ± 13.7	10.6 ± 3.8	0.14 ± 0.05	1,274.9 ± 269.0	4.62 ± 0.98
N = 11			Female body condition gains, *extrapolated to approx. date of birth*[3]	62.5 ± 10.9	17.2 ± 3.0	0.23 ± 0.04	1,691.5 ± 246.0	6.26 ± 0.90
			Average Daily Metabolic Rate (ADMR)[2]	---	---	---	14,302.4 ± 284.4	52.2 ± 0.9
	a;b		Foetal & Placental Tissue[4]	31.8 ± 0.6	8.7 ± 0.2	0.12 ± 0.01	271.7 ± 4.8	1.14 ± 0.02
	c		Heat Increment of Gestation (HIG)[5]	---	---	---	1,017.5 ± 25.0	3.78 ± 0.10
			Summed: female body condition gains at date of birth, with demand of ADMR, pup, placenta, & HIG	94.2 ± 11.1	25.8 ± 3.1	0.35 ± 0.04	17,473.6 ± 474.4	63.4 ± 1.6
			Difference between reproductive and non-reproductive females[6]	42.6	10.3	0.16	2,067.6	7.4
			Percent difference between reproductive and non-reproductive	82.6	66.5	84.2	13.4	13.2
N. elephant seal								
Non-Reproductive	*d*	200.2 ± 16.3	Female body condition gains[1]	158.9 ± 15.6	59.1 ± 5.4	0.80 ± 0.06	2,863.1 ± 274.8	14.6 ± 1.32
N = 17			Average Daily Metabolic Rate (ADMR)[2]	---	---	---	9,666.8 ± 1,013.5	47.0 ± 1.42
			Summed: female body composition change, with demand of ADMR	158.9 ± 15.6	59.1 ± 5.4	0.80 ± 0.06	12,529.9 ± 1,215.4	61.6 ± 2.28
Reproductive	*d*	224.6 ± 0.5	Female body condition gains, ~9.5 days post-partum[1]	195.7 ± 3.0	70.5 ± 1.2	0.84 ± 0.01	3,649.3 ± 72.9	15.6 ± 0.31
N = 109			Female body condition gains, *extrapolated to approx. date of birth*[3]	235.1 ± 3.1	84.5 ± 1.3	1.05 ± 0.01	3,899.3 ± 73.0	17.4 ± 0.32
			Average Daily Metabolic Rate (ADMR)[2]	---	---	---	11,733.2 ± 92.8	52.2 ± 0.41
	b; e		Foetal & Placental Tissue[4]	44.8	16.2 ± 0.2	0.20 ± 0.01	388.4	1.73 ± 0.01
			Heat Increment of Gestation (HIG)[5]	---	---	---	1,541.0	6.87 ± 0.02
			Summed: female body condition gains at date of birth, with demand of ADMR, pup, placenta, & HIG	279.9 ± 3.1	100.7 ± 1.4	1.25 ± 0.01	17,561.8 ± 137.0	78.2 ± 0.61
			Difference between reproductive and non-reproductive females[6]	121.0	41.6	0.45	5,031.9	16.6
			Percent difference between reproductive and non-reproductive	76.1	70.4	56.3	40.2	26.9

Table 3.3., *continued.*

Grey seal								
Reproductive	*f*	222.1 ± 3.0	Female body condition gains, *extrapolated to approx. date of birth*[1]	74.6 ± 6.7	56.8 ± 5.9	0.34 ± 0.03	2,002.2 ± 191.2	9.0 ± 0.82
N = 15			Average Daily Metabolic Rate (ADMR)[2]	---	---	---	6,221.4 ± 145.8	28.1 ± 0.7
	f; g		Foetal & Placental Tissue[4]	17.2	11.5 ± 0.4	0.07 ± 0.01	98.4	0.47
			Heat Increment of Gestation (HIG)[5]	---	---	---	488.3	2.14
			Summed: female body condition gains at date of birth, with demand of ADMR, pup, placenta, & HIG	91.8 ± 6.7	69.7 ± 6.2	0.41 ± 0.03	8,810.2 ± 275.1	39.7 ± 1.10

a) Wheatley et al. 2006; b) Lavigne & Stewart 1979; c) Brody 1945; d) modified from Robinson et al. 2012; e) Kretzmann, Costa & Le Boeuf 1993; f) Beck et al. 2003; g) Anderson & Fedak 1987

[1]Energy content of tissues was calculated as 37.33 $kJ \cdot g^{-1}$ lipid, and lean mass being comprised of 27% protein with 23.5 $kJ \cdot g^{-1}$ protein (Crocker et al. 2001).

[2]ADMR ($MJ \cdot day^{-1}$) was calculated as $2(70M^{0.75})$ with M being mass in kg (averaged between post-molt and pre-breeding values; Lavigne et al. 1982) and converted from kcal to MJ. ADMR was multiplied by the number of days between animal handlings for total MJ Demand.

[3]Assumed females lose 10.53 g mass·post-partum $kg^{-1} \cdot day^{-1}$ (Wheatley et al. 2006), with the same body composition as was measured ~7 days post-partum. One female was weighed just after birth by collaborators (Drs. Jay Rotella and Robert Garrott) and was of known mass. In northern elephant seals, mass was extrapolated to date of parturition using $(kg \cdot day^{-1})=0.51+0.0076 \cdot$post partum mass (Simmons et al. 2010).

[4]Five Weddell seal pups were weighed by collaborators within 3 days post-partum (umbilical cord present; Drs. Jay Rotella and Robert Garrott). All other Weddell seal pups were assumed to weigh 27.2 kg at birth, with body composition of 8.6% lipid (Wheatley et al. 2006). Neonate mass in northern elephant seals and grey seals was used from the literature (Kretzmann et al. 1993, and Anderson & Fedak 1987, respectively). Estimated placental mass is 12% of neonatal mass, with energy content of 4.79 $MJ \cdot kg^{-1}$ in all species (Lavigne & Stewart 1979).

[5]Heat increment of gestation (HIG) was calculated as $Q=4400M^{1.2}$, where Q is energy in kcal and M is neonate mass (kg) at birth. This was converted from kcal to MJ.

[6]Difference needed for gestation to bring a pup to term assumed to be the difference between: Reproductive female mass or energy gain − Non-reproductive female mass or energy gain. The percent difference shows the relative amount that seals must increase weight and energy accumulation (absolute and rate) over the winter relative to non-gestating seals.

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Chapter 4. Scaling Matters: Incorporating Body Composition into Weddell Seal Seasonal Oxygen Store Comparisons Reveals Maintenance of Aerobic Capacities[1]

4.1 Abstract

Adult Weddell seals (*Leptonychotes weddellii*) haul-out on the ice in October/November (austral spring) for the breeding season and reduce foraging activities for ~4 months until their molt in the austral fall (January/February). After these periods, animals are at their leanest and resume actively foraging for the austral winter. In mammals, decreased exercise and hypoxia exposure typically leads to decreased production of O_2-carrying proteins and muscle wasting, while endurance training increases aerobic potential. To test whether similar effects were present in marine mammals, this study compared the physiology of 53 post-molt female Weddell seals in the austral fall to 47 pre-breeding females during the spring in McMurdo Sound, Antarctica. Once body mass and condition (lipid) were controlled for, there were no seasonal changes in total body oxygen (TBO_2) stores. Within each season, hematocrit and hemoglobin values were negatively correlated with animal size, and larger animals had lower mass-specific TBO_2 stores. But because larger seals had lower mass-specific metabolic rates, their calculated aerobic dive limit was similar to smaller seals. Indicators of muscular efficiency, myosin heavy chain composition, myoglobin concentrations, and aerobic enzyme activities (citrate synthase and β-hydroxyacyl CoA dehydrogenase) were likewise maintained across the year. The preservation of aerobic capacity is likely critical to foraging capabilities, so that following the molt Weddell seals can rapidly regain body mass at the start of winter foraging. In contrast, muscle lactate dehydrogenase activity, a marker of anaerobic metabolism, exhibited seasonal plasticity in this diving top predator and was lowest after the summer period of reduced activity.

[1] Shero, M.R., D.P. Costa, and J.M. Burns. 2015. Scaling Matters: Incorporating body composition into Weddell seal seasonal oxygen store comparisons reveals maintenance of aerobic capacities. Journal of Comparative Physiology B, DOI: 10.1007/s00360-015-0922-8.

4.2 Introduction

The proficiency with which marine mammals can forage depends, in part, on their ability to carry large endogenous oxygen (O_2) stores (Scholander 1940; Hochachka and Storey 1975; Kooyman et al. 1980; Butler and Jones 1997; Kooyman and Ponganis 1998; Costa and Sinervo 2004; Burns et al. 2007). In deep diving phocid seals, lung collapse at depth ultimately renders this store inaccessible (Falke et al. 1985; Kooyman and Ponganis 1998) so the majority (~80%) of the O_2 stores are located in the blood and muscle tissues (Lenfant et al. 1970; Kooyman and Ponganis 1998). Moreover, pinnipeds are adapted to utilize O_2 stores efficiently, comprising the graded dive response. At the onset of a deep dive, seals exhibit bradycardia and employ peripheral vasoconstriction to apportion blood O_2 stores to specific tissues (Butler and Jones 1997; Guyton et al. 1995; Davis et al. 2004), such as the heart, lung, and brain (Zapol et al. 1979). Diverting blood O_2 stores primarily to anoxia intolerant organs means that skeletal muscles are often un-perfused (Zapol et al. 1979; Guyton et al. 1995; Davis et al. 2004; Williams et al. 2011). To withstand this reduction of vascular O_2 supply during periods of underwater activity, pinniped skeletal muscles have 10-20 fold the amount of myoglobin (Mb) compared to terrestrial mammals (Reed et al. 1994; Guyton et al. 1995; Kanatous et al. 1999; Polasek et al. 2006).

Large aerobic capacities and reduced O_2 consumption allow pinnipeds to delay the production of lactate, thereby extending the aerobic dive limit (ADL) (Kooyman et al. 1980; 1983). The ADL can be estimated by dividing endogenous O_2 stores by diving metabolic rates (DMR), which is an accurate estimator of the diving lactate threshold in Weddell seals (Kooyman et al. 1980; 1983; Ponganis et al. 1993; Burns and Castellini 1996; Costa et al. 2001). The diving lactate threshold, or the "true" ADL, will vary on a dive-by-dive basis depending on O_2 saturation at the start of a dive, the magnitude of the dive response, and metabolic rates (Castellini et al. 1992b; Guyton et al. 1995; Davis and Kanatous 1999). However, given the difficulty of measuring the diving lactate threshold in free-living animals, the calculated (c)ADL is used as a proxy (Costa et al. 2001; 2004; Costa and Sinervo 2004).

Knowing when lactate accumulates above background levels has ecological significance because in many species, if the (c)ADL threshold is exceeded, the post-dive recovery period increases

exponentially as lactate is cleared from the blood before the next dive (Kooyman et al. 1980). Alternatively, animals can continue diving despite high circulating lactate levels, but subsequent dives will be relatively short and aerobic until lactate levels decline (Castellini et al. 1988; Thompson and Fedak 1993). This ultimately decreases the total amount of time that can be spent foraging (Castellini et al. 1992b; Costa et al. 2001), and therefore, free-ranging pinnipeds are thought to limit the frequency of dives that exceed their ADL (Kooyman et al. 1980; Thompson and Fedak 2001). Because TBO_2 stores generally exhibit a linear relationship with body mass ($Mass^{1.0}$) whereas O_2 is depleted as an allometric function of $Mass^{0.75}$ (Kleiber 1947; 1975; Kooyman 1989), larger animals tend to have disproportionately longer ADL times (Schreer and Kovacs 1997; Halsey et al. 2006a; Halsey et al. 2006b). Further, O_2 is only stored in the lean body compartment, and therefore, the relationship of physiological parameters with animal size is complicated by extreme seasonal fluctuations in lipid stores (Costa et al. 1986; Wheatley et al. 2006; McDonald et al. 2008; Crocker et al. 2014).

In addition to animal mass and body composition (lipid stores), activity patterns may also have an impact on the ADL. This is because the production of many O_2 storage proteins is facilitated by exercise and hypoxia exposure (Hochachka et al. 1998; Hoppeler and Vogt 2001; Halvorsen and Bechesteen 2002; Haddad et al. 2003), both of which stimulate the NFAT/MEF-2, hypoxia inducible factor (HIF)-1, and Sp1 pathways to increase transcription of Mb, glycolytic enzymes, erythropoietin (EPO), and vascular endothelial growth factor (VEGF)-1 (Hochachka and Somero 2002, Kanatous and Mammen 2010; De Miranda et al. 2012). Weddell seals (*Leptonychotes weddellii*) likely reduce their diving activity during their breeding and molting periods, as do other phocid species (Kooyman 1975; Castellini et al. 1992a; Schreer and Testa 1996; Forcada et al. 2012). If cellular pathways regulating the production of O_2-storage proteins are down-regulated during this period, it would likely negatively impact foraging success, as O_2 stores and the ADL may be reduced.

In addition to having large O_2 stores, pinniped muscles must be able to generate sufficient power while consuming minimal O_2 during dives (Davis et al. 2004). The generation of muscle force for propulsion is largely determined by muscle fiber and myosin heavy chain (MHC) isoform composition. Endurance training leads to an increase in slow-twitch oxidative (SO) fiber types with predominately MHC I protein, high mitochondrial densities, elevated Mb loads, and greater

aerobic enzyme activities such as citrate synthase (CS; initiates the TCA cycle) and β-hydroxyacyl CoA dehydrogenase (HOAD; in the β-oxidation chain for fatty-acids), and pinnipeds have muscular profiles reflecting oxidative and endurance potential (Kanatous et al. 2002; Luedeke et al. 2004). Yet, SO fibers are the most vulnerable to atrophy during times of disuse (Booth 1977; 1982; Hudson and Franklin 2002). Muscles that can generate high force with rapid contractions are primarily composed of fast-twitch oxidative-glycolytic (FOG) fibers, containing MHC IIA and MHC IID/X (Baldwin and Haddad 2001). Muscles with primarily FOG fibers express greater lactate dehydrogenase (LDH) activities, so that they can rapidly convert pyruvate to lactate under low O_2 and burst-speed conditions (Peter et al. 1972; Baldwin et al. 1973; Kanatous et al. 2002). Because muscle mass and function are energetically costly to maintain, mammalian skeletal muscles show large capacities for phenotypic plasticity (Baldwin and Haddad 2001; Hoppeler and Flück 2002; Luedeke et al. 2004; Flück 2006), and are particularly affected by hormones, hypoxia, and exercise regimens (Stockdale and Miller 1987; Hoppeler and Flück 2002; Luedeke et al. 2004; Flück 2006).

This study aimed to determine whether the aerobic capacity and muscular efficiency of adult female Weddell seals is reduced following the pupping and molting periods when seals have limited their underwater activities. Therefore, TBO_2 stores, as well as muscle structural and biochemical properties were measured directly post-molt and compared to those at the start of the summer breeding period, after intense winter foraging. We also determined whether females that returned to give birth after the austral winter foraging period had longer cADL times or muscle profiles indicative of greater power and efficiency, than those animals that returned but failed to produce a pup.

4.3 Methods

4.3.1 Animal Handling

Fifty-three adult female Weddell seals were captured on the ice along the McMurdo Sound region, Antarctica in Erebus Bay (~77°S, 165°E) and the Victoria Land coastline (~76°S, 162°E) following the molt in January/February (austral fall), and 47 females (30 non-reproductive

females; 17 reproductive females handled 7 days post-partum) were handled in October/November (austral spring) 2010-2012, following the winter foraging period. These animals comprise the large cross-sectional portion of this study. Of the Weddell seals handled in spring (year t+1), 20 were animals that had been handled the previous fall (year t), and these animals constitute the longitudinal portion of this study. Twelve of these recaptured females returned with a pup the following year (t+1), and eight animals did not give birth after the winter foraging period. All the post-molt animals were assumed to be non-reproductive because none of the molted known-age individuals handled and < 15% of fully molted females surveyed in the population in fall had given birth in the pupping period immediately preceding sampling (Burns et al. 2013; Beltran and Burns *unpublished*).

Animals were sedated with an initial intramuscular dose of ~1.0 $mg \cdot kg^{-1}$ tiletamine/zolazepam HCl. Following a 10 to 15 minute induction period, animals were captured via hoop net and additional intravenous injections of ketamine and diazepam (~0.2 $mg \cdot kg^{-1}$ and 0.012 $mg \cdot kg^{-1}$) were administered approximately every 10 mins, or as necessary, to keep animals sedated while remaining eupneic. A straight length was taken and total body mass (TBM) was measured using a sling with a hand winch and scale (MSI-7200-IT Dyna-Link digital dynamometer, capacity 1,000 ± 1.0 kg) while animals were suspended from a tripod. Body composition (%lipid) and lean body mass (LBM) was determined for each animal using tritiated water dilution as described in Shero et al. (2014; 2015) (Table 4.1).

4.3.2 Hematology & Blood Volume

Blood samples were collected in EDTA and heparinized vacutainers™ from the extradural vein. Hematocrit (Hct; packed RBC volume) was determined by whole blood centrifugation, and hemoglobin (Hb) concentrations ($g \cdot dL^{-1}$) were determined for each animal using the cyanomethemoglobin assay with Drabkin's reagent (Sigma Kit 625A) and a UV/Vis Beckman series 530 spectrophotometer (Beckman Coulter, Inc., Fullerton, CA) at λ=540 nm and concentrations of samples calculated through a linear regression of hemoglobin standards (Pointe Specific, Inc.). Mean corpuscular hemoglobin concentration (MCHC) was calculated as:

$$\text{(Eq. 4.1)} \qquad \text{MCHC}(\%) = \frac{\text{Hb g} \cdot \text{dL}^{-1}}{\text{Hct}} \times 100.$$

Medix^{TM} Ery-Tic RBC test kits were used to count red blood cells (RBCs) (Medix Corp., Newbury Park, CA).

Plasma volume (in liters; PV_L) was determined using the Evan's Blue technique. One pre-injection blood sample was taken followed by administration of ~0.5-1.2 mg·kg^{-1} Evan's Blue dye into the extradural vein. Syringes used to distribute the dye were pre-weighed and flushed with blood to accurately determine the amount of dye injected for each animal. Injection was followed by three consecutive blood draws approximately 10 min apart, and the exact time of sample collections recorded. Blood samples were centrifuged and plasma stored at -80°C until analyses. The plasma background absorbance values were measured at λ=740 nm and were subtracted from optical density at λ=624 nm. Evan's Blue dye stock (40 mg·mL^{-1}) was used to construct standard curves and determine sample concentrations. Dilution of the dye was used to calculate PV_L as described in Foldager and Blomqvist (1991) and El-Sayed et al. (1995) and blood volume (in liters; BV_L) calculated as:

$$\text{(Eq. 4.2)} \qquad BV_L = \frac{PV_L}{(100\text{-Hct}) / 100.}$$

4.3.3 Myoglobin Concentrations

Longissimus dorsi (*LD*) skeletal muscle samples were taken from each animal in the field with a 6 mm biopsy punch and immediately frozen in liquid nitrogen before being stored at -80°C until analysis. Muscle Mb concentrations (mg·g wet tissue^{-1}) were assayed following Reynafarje (1963) as modified by Prewitt et al. (2010). Samples were run in quadruplicate in a Molecular Devices SpectraMax 340 microplate reader (Molecular Devices, Inc., Sunnyvale, CA) alongside a lyophilized myoglobin horse standard (Sigma, mean recovery: 100.6 ± 2.82%) and previously assayed harbor seal (*Phoca vitulina*) and Weddell seal tissue (Inter-assay CVs: harbor seal 6.5%; Weddell seal 4.6%). Samples were read at both λ=538 and 568 nm to account for any Hb contamination.

4.3.4 Total Body Oxygen Stores & the cADL

Blood O_2 stores were determined from Hb, Hct, and BV in arterial and venous systems with the following assumptions: 1) hemoglobin has an O_2 carrying capacity of 1.34 mL $O_2 \cdot g^{-1}$ [Hb], 2)

arterial blood is 33% of total blood volume, with the remaining 66% blood in the venous system, 3) the maximum O_2 saturation possible in the arterial system is 95%, with a minimum of 20% after O_2 has been transported to other tissues, and 4) venous blood is presumed to have 5% less volume than the starting arterial O_2 stores and can be extracted to zero (Kooyman et al. 1983; Davis and Kanatous 1999; Burns et al. 2007). Muscle O_2 stores were determined for each animal assuming that Mb also had an O_2 carrying capacity of 1.34 mL $O_2 \cdot g^{-1}$ [Mb] and total muscle mass was estimated as 38% of lean body mass (Burns et al. 2007). Lung O_2 stores were calculated as:

(Eq. 4.3) $\quad \text{Lung } O_2 = V_i \times 0.15\ FO_2.$

V_i is the estimated diving lung volume (in liters) calculated as $0.5 \times 0.10(\text{TBM})^{0.96}$, which assumes that lung volume is at 50% total capacity at the onset of diving. Further, 0.15 FO_2 is the partial pressure of O_2 in the lungs (Kooyman 1989). Blood, muscle, and lung O_2 stores were summed to give total body oxygen (TBO_2) stores of animals for which all measurements were possible (Lenfant et al. 1970; Kooyman et al. 1983; Burns et al. 2007). Each animal's diving metabolic rate (DMR) was estimated from 1.6 × Kleiber (Kleiber 1975; Williams et al. 2004). Calculated aerobic dive limits (cADL) were determined by dividing TBO_2 stores by DMR, and TBO_2 stores were scaled to TBM and LBM for seasonal comparisons.

4.3.4 Enzyme Kinetics

To evaluate aerobic and anaerobic ATP production potential, citrate synthase (CS), β-hydroxyacyl CoA dehydrogenase (HOAD), and lactate dehydrogenase (LDH) kinetic activities (IU$\cdot g^{-1}$ wet mass muscle) were measured. Spectrophotometric assays were run according to the procedures described by Polasek et al. (2006) and Prewitt et al. (2010) under substrate saturating conditions held at 37°C. A buffer blank and a previously assayed muscle sample of known concentration were measured as controls along with all experimental samples (Inter-assay CVs: CS: 13.1%, HOAD: 10.4%, LDH: 7.0%). CS:HOAD ratios were evaluated to determine the relative amount of aerobic metabolism that utilizes β-oxidation of fatty acids. Values less than 1 indicate higher dependence on lipid stores. The LDH:CS ratios were calculated to compare potentials for aerobic versus anaerobic metabolism (Polasek et al. 2006).

4.3.5 Myosin Heavy Chain Isoform Composition

Myosin heavy chain isoforms were separated using the SDS-PAGE technique as described by Blough et al. (1996) and Reiser and Kline (1998). Weddell seal samples were run in triplicate against a rat (*Rattus norvegicus*) sample that contained MHC I, IIA, IIB, and IID/X (50:50 soleus: extensor digitorum longus) as a standard. Gels were silver-stained and developed as described by Blough et al. (1996) and bands were quantified using digitizing software (UN-SCAN IT gel v. 6.1). The identity of Weddell seal MHC I, IIA, and IID/X bands was determined by comparing their relative migration distances to those in the rat standard, and those of other pinnipeds that had previously been confirmed by proteomic analysis (Shero et al. 2012) at the Ohio State University Mass Spectrometry and Proteomics Facility. New proteins were identified using capillary-liquid chromatography tandem mass spectrometry (Cap-LC/MS/MS) using a Thermo Scientific LTQ mass spectrometer (Bruker Daltonics Billerica, MA) and LC system (UltimMateTM 3000 System, Thermo Scientific). Dynamic exclusion was enabled on the mass spectrometer with two repeats within ten seconds, a mass list size of 200, exclusion duration of 350 seconds, and a low mass width of 0.5 and high mass width of 2.5. A Mascot Daemon (Matrix Science v. 2.3.2, Boston, MA) search against the SwissProt Database was performed to identify significant protein matches. Two missed cleavages for the enzyme were permitted.

4.3.6 Statistical Analyses

Data were assessed for normality prior to statistical analysis, and transformed as necessary. To determine whether physiology differed seasonally and for animals that were in better condition (more lipid) for their mass, linear mixed models (LMMs) with Bonferroni post-hoc comparisons were used with reproductive category as a fixed factor (fall non-reproductive, spring non-reproductive, spring reproductive), and total body mass (TBM) and body composition as covariates in models. Repeated measures were used to account for the fact that some individuals were handled in both fall and spring. Model residuals were evaluated for normality and homoscedasticity, and standardized residuals were used to evaluate outliers. Because MHC profiles were not normally distributed even after transformation, a Kruskal-Wallis H test was used to test for differences in MHC composition by reproductive group (SPSS v. 22, Chicago, IL, USA). Regression analyses were used to assess relationships between MHC profiles with

animal condition and muscle biochemical properties. Overwinter changes in physiological parameters of females handled in both fall (year t) and spring (t+1) were analyzed using paired t-tests. Significance was defined at the 95% level ($P<0.05$) throughout, and results are reported as mean ± SE.

4.4 Results

4.4.1 Hematology & Blood Volume

In the cross-sectional study, blood Hct, Hb, and MCHC were negatively correlated with TBM (Table 4.2; Hct: $F_{1, 75.4} = 11.8$, $P = 0.001$; Hb: $F_{1, 73.1} = 20.7$, $P < 0.001$; MCHC: $F_{1, 44.6} = 5.0$, $P = 0.031$) and body composition did not significantly improve model fit. While Hb (24.7 ± 0.3 $g \cdot dL^{-1}$) and MCHC (40.1 ± 0.3%) did not vary by reproductive status, Hct was significantly lower in spring reproductive females than non-reproductive females in both fall and spring (Table 4.2; $F_{2, 87.7} = 6.8$, $P = 0.002$). RBC numbers did not correlate with animal size or body composition, but spring reproductive females had significantly lower RBC counts than non-reproductive females in the fall ($F_{2, 33.8} = 4.7$, $P = 0.016$). In the smaller longitudinal study, Hct and Hb declined slightly as animals gained mass overwinter, but these trends were not significant as in the larger dataset. However, MCHC decreased significantly overwinter in both nonparous and parous females in the longitudinal study (non-parous: $t_5 = 3.9$, $P = 0.011$, parous females: $t_{10} = 2.6$, $P = 0.024$).

PV_L was significantly positively correlated with TBM and adding body composition significantly improved the model (Fig. 4.1A-B; Table 4.2; TBM: $F_{1, 80.4} = 623.4$, $P < 0.001$; Body Composition: $F_{1, 86.2} = 27.9$, $P < 0.001$). Non-reproductive females in spring had significantly higher plasma volume than reproductive females, but neither group differed from non-reproductive females in fall ($F_{2, 80.6} = 8.4$, $P < 0.001$). BV_L also exhibited a significant positive relationship with TBM (Fig. 4.1C-D; TBM: $F_{1, 81.3} = 254.4$, $P < 0.001$), and appeared to differ by reproductive group with fall non-reproductive females having the highest blood volume per unit body mass (Table 4.2). But this effect was an artifact of seasonal differences in body composition (spring > fall), as animals with lower than expected BV_L for their size were

significantly fatter ($F_{1, 86.6} = 6.4$, $P = 0.013$). Similarly, blood O_2 stores scaled positively with TBM (Table 4.2; $F_{1, 84.9} = 56.6$, $P < 0.001$), yet models were not improved with the addition of body composition or reproductive group (14.3 ± 0.3 L O_2). In the longitudinal study, neither BV_L nor PV_L differed by season and mass-specific blood O_2 stores (scaled to TBM and LBM) did not change overwinter.

4.4.2 Muscle Biochemistry & Structure

In the large cross-sectional study, neither reproductive group, TBM, or body composition accounted for significant variation in muscle Mb or aerobic enzyme activities (Table 4.3; overall averages Mb: 90.0 ± 1.7 mg·g wet tissue^{-1}; HOAD: 23.2 ± 0.8 IU·g wet tissue^{-1}; CS: 14.4 ± 0.4 IU·g wet tissue^{-1}). The CS:HOAD ratio showed a significant negative relationship with body composition ($F_{1, 75.8} = 5.4$, $P = 0.023$), but not reproductive group or TBM. Among females that were captured in both fall (year t) and the subsequent spring (t+1), Mb was maintained. Females that were reproductive in year t+1 exhibited slight, but non-significant, decreases in CS and increases in HOAD, which together led to a significant decrease in the CS:HOAD ratio ($t_9 = 3.751$, $P = 0.005$). In the large cross-sectional study, muscle O_2 stores increased with TBM and the addition of body composition significantly improved model fit (Table 4.4; TBM: $F_{1, 74.3} = 113.0$, $P < 0.001$; Body composition: $F_{1, 78.9} = 11.2$, $P = 0.001$). Muscle O_2 stores did not differ by reproductive group (10.4 ± 0.3 L O_2). Similarly, muscle O_2 stores, whether scaled to TBM or LBM, in the smaller longitudinal study did not vary seasonally.

LDH activities and LDH:CS ratios were significantly positively correlated with TBM (Table 4.3; LDH: $F_{1, 75.5} = 10.5$, $P = 0.002$; LDH:CS ratio: $F_{1, 74.2} = 4.4$, $P = 0.040$), but the addition of body composition did not improve model fit. In contrast to indicators of aerobic metabolism, LDH activities and LDH:CS ratios differed by reproductive group (LDH: $F_{2, 57.3} = 13.5$, $P < 0.001$; LDH:CS ratio: $F_{2, 68.0} = 4.7$, $P = 0.012$) and non-reproductive females in spring had significantly higher LDH activities and LDH:CS ratios than post-molt seals in fall (LDH: $P < 0.001$; LDH:CS ratio: $P = 0.015$) but neither group differed from spring reproductive females. While females handled in both seasons showed a similar increase in LDH over winter as in the large cross-sectional dataset, the increase was not significant.

We identified four different MHC isoforms in Weddell seal *LD* muscle. MHC I and IIA were the two most abundant myosin isoforms, with MHC IID/X only present in six samples (Fig. 4.2). An additional MHC band was detected in six Weddell seals. Because this new MHC was very faint in Weddell seal muscle samples, not enough protein could be collected for proteomic analysis. However, this band migrated the same distance as one detected in greater concentration in hooded seal (*Cystophora cristata*) muscle. When this MHC band was sent out for proteomic analysis, a wide range of MHC isoforms were identified as close matches in the MASCOT and SwissProt database, making it difficult to determine its functional role (Closest matches: *1)* Accession number Q076A4, canine MYH8, slow-oxidative perinatal, *2)* Accession number Q076A5, canine MYH4, fast-glycolytic MHC IIB, *3)* Accession number Q8MJV0, horse MYH1, fast oxidative-glycolytic MHC IID/X, and *4)* Accession number P49824, canine MYH7, slow-oxidative cardiac MHC beta). The number of unique peptide sequence matches ranged from 31 to 64, and Molecular Weight (MOWSE) scores to the unidentified band ranged from 531 to 1,254 (scores >67 are significant; Pappin et al. 1993).

There were no differences in the relative proportion of either MHC I or IIA among reproductive groups (I: $\chi^2 = 2.9$, $P = 0.230$; IIA: $\chi^2 = 2.8$, $P = 0.244$) nor was MHC composition correlated with TBM or body composition. However, the MHC proportions were highly variable among individuals (range MHC I: 32.5-100%; range MHC IIA: 0-66.0%), and the variation was correlated with several measured muscle biochemistry parameters. For example, the relative proportion of MHC I was positively correlated with muscle Mb concentrations and HOAD activities (Fig. 4.3A-D; Mb: $F_{1, 88} = 28.9$, $P < 0.001$; HOAD: $F_{1, 83} = 12.0$, $P < 0.001$), while the relative proportion of MHC IIA was negatively correlated with the same parameters. In the longitudinal study, MHC composition remained constant in some recaptured animals while changing markedly in others, but there was no clear pattern with any measured variable.

4.4.3 Total Body Oxygen Stores & the cADL

Total body O_2 stores were significantly positively correlated with TBM (Fig. 4.4A; $F_{1, 74.1} = 223.3$, $P < 0.001$) and when considered alone, the relationship differed by reproductive group. However, females with lower TBO_2 stores for their size had significantly greater lipid stores (Fig. 4.4B; $F_{1, 79.1} = 5.7$, $P = 0.020$), and once both size and body composition were accounted

for, there were no differences in TBO_2 stores among reproductive groups (Table 4.4; 26.5 ± 0.6 L O_2). The slope of the relationship between TBO_2 and TBM was significantly lower than the overall mean mass-specific TBO_2 store value (slope: 65.9 mL $O_2 \cdot kg^{-1}$, 95% CI: 57.1 – 74.6 mL $O_2 \cdot kg^{-1}$; study mean: 78.9 ± 1.2 mL $O_2 \cdot kg^{-1}$), indicating that larger animals had significantly lower TBO_2 per unit body mass than smaller seals.

Once TBO_2 stores were divided by the estimated DMR, cADL times were not correlated with TBM (Table 4.4; Fig. 4.4C). When body composition was included in models, condition accounted for a significant amount of variation in cADL times (Fig. 4.4D; $F_{1, 78.9} = 6.9$, $P = 0.010$) and animals with shorter than expected cADLs were in better condition. cADL times differed by reproductive group ($F_{2, 78.5} = 3.2$, $P = 0.047$), and non-reproductive females in fall had significantly higher cADLs than non-reproductive females in spring ($P = 0.041$). There were no overwinter changes in the cADL of females handled in both fall (year t) and the subsequent spring (t+1), regardless of whether they pupped or not.

4.5 Discussion

This study shows that despite the seasonal changes in Weddell seal activity budgets (Kooyman 1975; Castellini et al. 1992a; Schreer and Testa 1996; Forcada et al. 2012) and body condition (Wheatley et al. 2006; Shero et al. 2015), their ability to sustain aerobic metabolism while underwater as indicated by TBO_2 stores, are conserved across the year. Variation in O_2 stores was most strongly correlated with animal mass, and not season. Similarly, the aerobic nature of muscle structure and biochemistry were stable across seasons and reproductive status. Maintenance of aerobic capacities across the year would allow animals to forage effectively at the end of the annual molt. Rapid reacquisition of tissue stores following lactation and molt is likely critical to maintenance of early gestation and future reproductive success (Smith 1966; Atkinson 1997). In contrast, the summer period of reduced diving activity was associated with a decline in LDH activity, as post-molt seals had the lowest LDH values of animals handled in this study. Overwinter increases in LDH may allow animals to extend dive durations even further, just prior to the pupping period the subsequent year.

This study found no evidence for seasonal declines in O_2-storage capabilities in this diving predator. In both fall and spring, Weddell seal hematological parameters were similar to values seen in previous studies (Hindle et al. 2011; Mellish et al. 2011). Only blood Hct and RBCs were lower in reproductive Weddell seals, and Hb and BV did not change seasonally. Muscle O_2 stores, as indicated by Mb concentrations, were higher than previously reported in Weddell seals (Ponganis et al. 1993; Kanatous et al. 2002; 2008; Hindle et al. 2011), but were also maintained across the year. As muscle Mb loads often correlate with dive durations (Noren and Williams 2000; Lestyk et al. 2009), higher Mb concentrations in deep-diving Weddell seals makes their O_2-storage capacities more similar to those previously reported in other phocid species capable of extended breath hold diving (Burns et al. 2007; Lestyk et al. 2009; Hassrick et al. 2010). That Weddell seals expend energy to continue producing equivalent RBCs and iron-containing O_2-carrying proteins during all times of the year (i.e., reduced and intense foraging periods, and energetically-expensive lactation and molt periods), and also through senescence (Hindle et al. 2009; 2011) demonstrates the importance of maintaining aerobic diving capacities. One possibility is that any summer diving activity and exercise would be sufficient to maintain protein production in Weddell seals. Pinnipeds also have sleep apneas while hauled-out and this may provide additional "hypoxic exposure," that may serve to maintain aerobic capacity during periods when underwater activity is limited (Castellini et al. 1992b; Castellini 1994; 1996; Zenteno-Savin and Castellini 1998). Alternatively, because the turnover time for RBCs and muscle Mb is ~2-4 months (Hickson and Rosenkoetter 1981; Ben-David and Flaherty 2012), the breeding and molt periods may not provide sufficient time to observe a decrease in protein production, before animals would need to prepare for active foraging again. In combination, due to the preservation of blood and muscle O_2 storage proteins across the year, TBO_2 stores were also maintained.

An important outcome of this work is that both mass and body composition need to be considered when comparing TBO_2 stores and cADL among individuals and/or species. Simply using mass as a scalar for O_2-stores ignores the dramatic variation in blubber and other lipid compartments that do not store O_2 but affect metabolism. When the relationship between TBO_2 and TBM was considered alone, it appeared as though animals had significantly lower TBO_2 stores, for their mass, in spring. However, when controlling for variation in lipid stores across individuals, TBO_2 stores did not differ by season. This comparison accounting for animal body

composition is most physiologically-relevant, as it tests whether there are differences in O_2-storage capacities in the lean body compartment. Weddell seals exhibited a relatively small over winter increase in body condition (lipid increased by 6%TBM) as compared to other marine mammal species and at different times of the year (i.e., lipid may decrease by 15%TBM across lactation; Costa et al. 1986; Beck et al. 2003; Wheatley et al. 2006; Crocker et al. 2014). Still, these small changes in lipid stores were enough to bias interpretations of seasonal differences in TBO_2 stores, before including body composition in statistical models. This further emphasizes the importance of incorporating body composition into TBO_2 store calculations. It should also be noted that because TBM was used to calculate lung O_2 stores, our estimate of TBO_2 stores is not entirely independent of TBM. However, lung O_2 stores only contribute ~ 7% to Weddell seal TBO_2 stores, and so any remaining bias is likely small.

In addition to changes in O_2 stores, changes in DMR have the potential to greatly alter the ADL both on an individual dive basis (Castellini et al. 1992b; Williams et al. 2004; Fahlman et al. 2008), as well as across the year (Gerlinsky et al. 2014). For example, cetaceans tend to have lower TBO_2 stores than phocid seals, but their large size is associated with low DMRs, extending their aerobic capacities (Noren and Williams 2000; Croll et al. 2001). Diving mammals can, conversely, also greatly exceed their ADLs to reach rich or ephemeral prey patches (Costa et al. 2001; 2004). The relationship between animal size, O_2 stores, and dive durations also varies by taxonomic family (Schreer and Kovacs 1997; Halsey et al. 2006a; Halsey et al. 2006b). For example, otariids tend to have shorter dive durations than phocids of comparable size, as do diving seabirds, due to lower O_2 stores and higher mass-specific metabolic rates. Within species, the ADL and dive durations increase during ontogeny (Kooyman et al. 1983; Burns and Castellini 1996; Richmond et al. 2006; Burns et al. 2007; Fowler et al. 2007; Weise and Costa 2007; Shero et al. 2012). However, among adults the relationship is less clear, and in this study larger Weddell seals had lower mass-specific TBO_2 stores than smaller seals. Lower estimated mass-specific metabolic rates ($DMR \propto Mass^{0.75}$) in larger animals then resulted in equivalent cADLs across size classes. The cADLs in this study were comparable to the "true" ADL with increased lactate concentrations occurring at 20-25 minutes in Weddell seals (Kooyman et al. 1980; Williams et al. 2004).

Despite evidence that protein expression of muscle Mb, enzymes, and structural components is controlled by different regulatory pathways (Jansson et al. 1988; Terrados et al. 1990), all aerobic biochemical properties of Weddell seal primary locomotor muscles remained constant across both seasons in this study. Weddell seal locomotor muscles contained primarily MHC I and IIA (Kanatous et al. 2002; 2008), and other MHC isoforms were rare and/or not present in all individuals. In terrestrial mammals, such as humans, mice and rats, and sled and raccoon dogs, numerous aerobic aspects of muscles atrophy quickly during periods of inactivity and food deprivation (i.e., myofibers atrophy, capillary and mitochondrial densities drop, Mb and aerobic enzyme activities decrease, and MHC shifts from slow to fast-types; Lindboe et al. 1982; Baldwin and Haddad 2001; Flück 2006; Gerth et al. 2009; Kinnunen et al. 2015). Conversely, in Weddell seals the great oxidative potential of muscles was reflected by stable CS and HOAD enzyme activities, in addition to high and relatively invariant Mb concentrations. These findings suggest that Weddell seals may be physiologically "programmed" to withstand periods of reduced activity while maintaining muscle integrity. Wild animals may need to forage effectively and escape predation after such periods of reduced activities, and indeed, similar patterns of atrophy resistance have been observed in hibernating bats and rodents, and winter lethargy in bears (Lohuis et al. 2007; Hershey et al. 2008; Lee et al. 2008; Nowell et al. 2011). Despite being comprised of primarily slow-oxidative fibers that are particularly vulnerable to atrophy, Weddell seal muscles maintained aerobic MHC profiles. Muscle structure was strongly correlated with many biochemical features, and these relationships persisted throughout the year, further suggesting that preservation of muscular force and efficiency is critical to foraging success in this species.

While physiological indicators of aerobic capacity and muscle structure were largely conserved, anaerobic capacity, as judged from muscle LDH activity, exhibited seasonal plasticity. As would be expected based on controlled cell culture experiments and detraining protocols (Semenza et al. 1994; Mujika and Padilla 2001; De Miranda et al. 2012), reduced activity and hypoxic exposure during the Weddell seal's summer breeding period and molt was associated with ~25% lower LDH activities, suggesting that glycolytic enzyme activities may atrophy faster than aeorbic enzymes. Because LDH was also positively correlated with animal size, animals were larger in spring with higher LDH activities. Higher glycolytic enzyme activities may allow animals to extend their dives to longer durations, past the cADL, more frequently in the spring.

Higher LDH activities would also clear lactate faster during surface recuperation periods by catalyzing its conversion to pyruvate (Thompson and Fedak 2001; Davis et al. 2004). The maintenance of aerobic capacity and increased anaerobic capacity across the winter may reflect a burst in foraging abilities prior to the pupping season the following year.

Studies conducted in other pinniped species have shown opposing results on seasonal changes in TBO_2 stores and the cADL. In multiple sea lion species, variation in blood and muscle O_2 stores emerged in response to changes in food availability and/or nutritional plane (Villegas-Amtmann and Costa 2010; Villegas-Amtmann et al. 2012; Gerlinsky et al. 2014). In contrast to otariids, the Phocidae family appears to follow a different pattern. In addition to TBO_2 and muscle biochemistry being conserved in Weddell seals, the northern elephant seal (*Mirounga angustirostris*) did not exhibit any changes in factors contributing to TBO_2 or the cADL across the post-molt or post-breeding foraging trips (Hassrick et al. 2010), and studies show that harbor seal (*Phoca vitulina*) muscle biochemistry does not change seasonally (Burns *unpublished*). Weddell seals and other phocids forage on unreliable prey resources spanning great distances in order to accumulate some or all of the reserves necessary for energetically-costly periods of reproduction and the annual molt (Costa and Shaffer 2012). Loss of body condition during these periods likely makes the initiation of winter foraging and early gestation a critical recuperation period for female Weddell seals to prepare for the next year. Maintaining high O_2 stores throughout the year would be imperative to effective foraging, and also to buffer times when prey is scarce due to environmental perturbations or other anomalies.

4.6 Acknowledgements

We thank field team members: Kimberly Goetz, Linnea Pearson, Dr. Patrick Robinson, and Dr. Luis Hückstädt for sample collection, and also group B-009-M led by Drs. Robert Garrott, Jay Rotella, and Thierry Chambert for their help locating study animals. Logistical support was provided by the National Science Foundation (NSF) U.S. Antarctic Program, Raytheon Polar Services, and Lockheed Martin ASC; we thank all the support staff in Christchurch, NZ and McMurdo Station. This research was conducted with support from NSF ANT-0838892 to D.P.C. and ANT-0838937 to J.M.B. This material is based upon work supported by the National

Science Foundation Graduate Research Fellowship (to M.R.S.) under Grant No. DGE-1242789. Any opinion, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation. Animal handling protocols were approved by the University of Alaska Anchorage and University of California Santa Cruz's Institutional Animal Care and Use Committees. Research and sample import to the United States was authorized under the Marine Mammal permit No. 87-1851-04 issued by the Office of Protected Resources, National Marine Fisheries Service. Research activities were approved through Antarctic Conservation Act permits while at McMurdo Station.

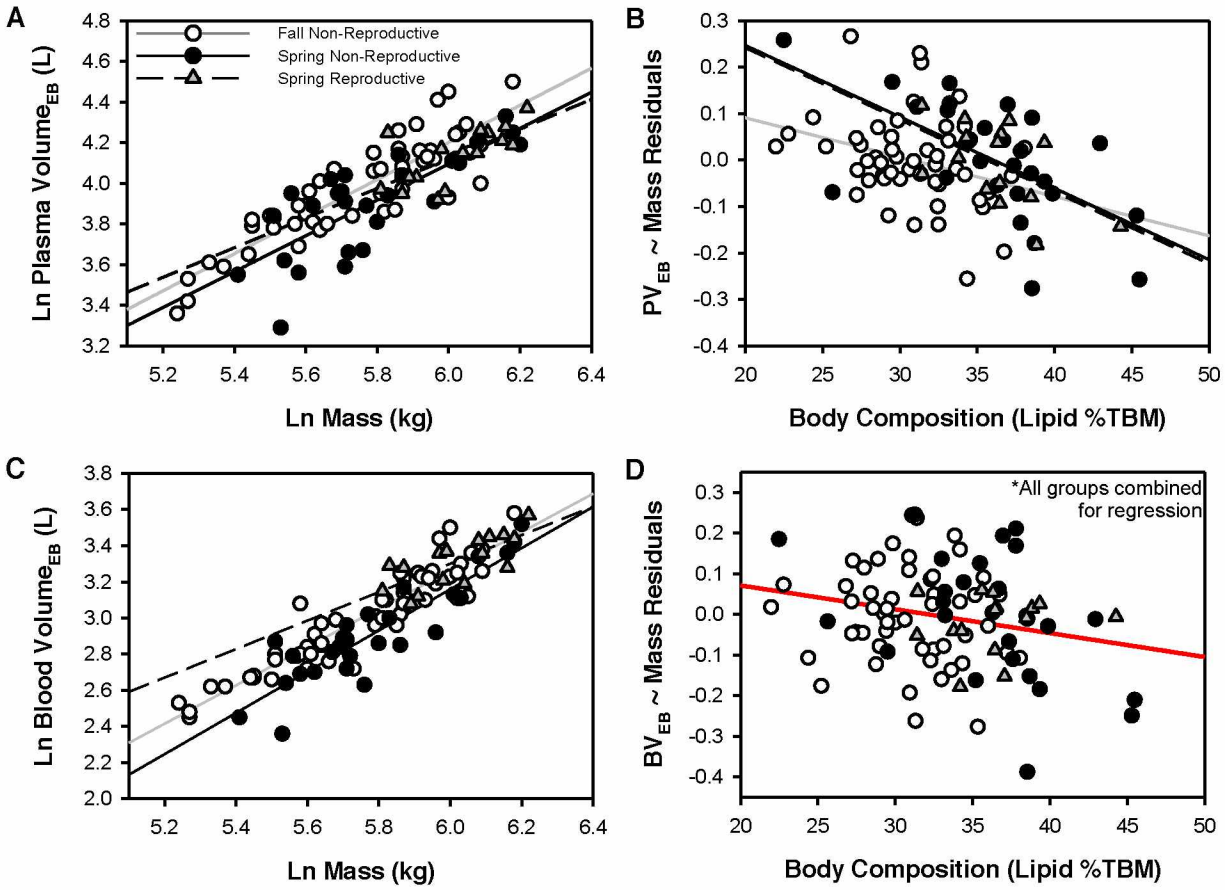

Figure 4.1. Relationships between Weddell seal plasma and blood volume with body mass and composition. Linear mixed models between Weddell seal plasma volume determined using Evan's blue dye and total body mass (**A, C**). The LMM residuals from plasma or blood volume regressed against total body mass, were significantly negatively correlated to body composition (**B, D**). Relationships differed by reproductive group in all LMM models (Fall Non-reproductive: *solid grey line*, Spring Non-reproductive: *solid black line*, Spring Reproductive: *dashed black line*), except for blood volume after body composition was accounted for (All reproductive groups combined: *single, thick solid red line*).

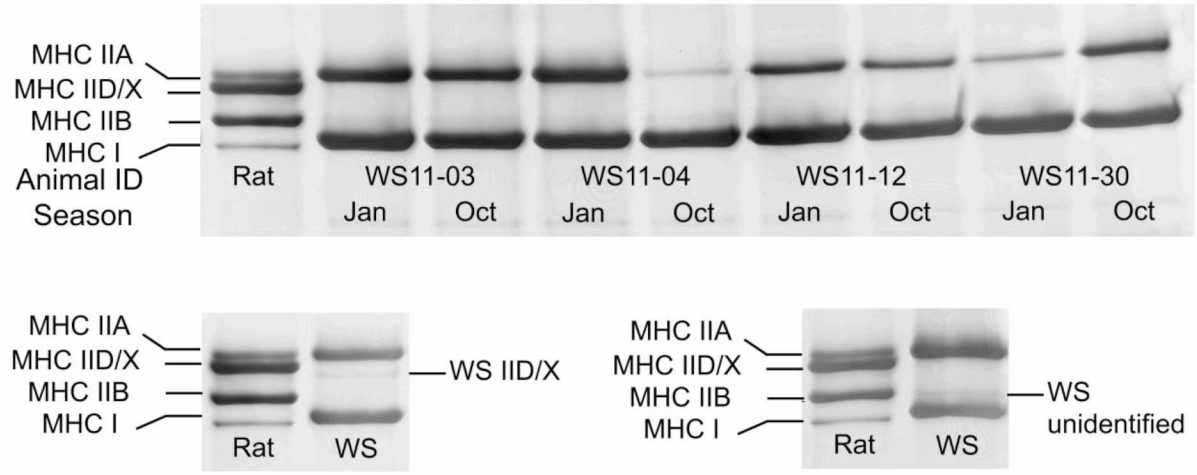

Figure 4.2. Weddell seal (WS) myosin heavy chain (MHC) gels. (*Above*) Muscle MHC composition for females that were handled at two different times of year. MHC I and IIA were dominant. (*Below*) A few animals had the fast-twitch oxidative-glycolytic MHC IID/X isoform, or the unidentified isoform. All samples were run against a rat standard.

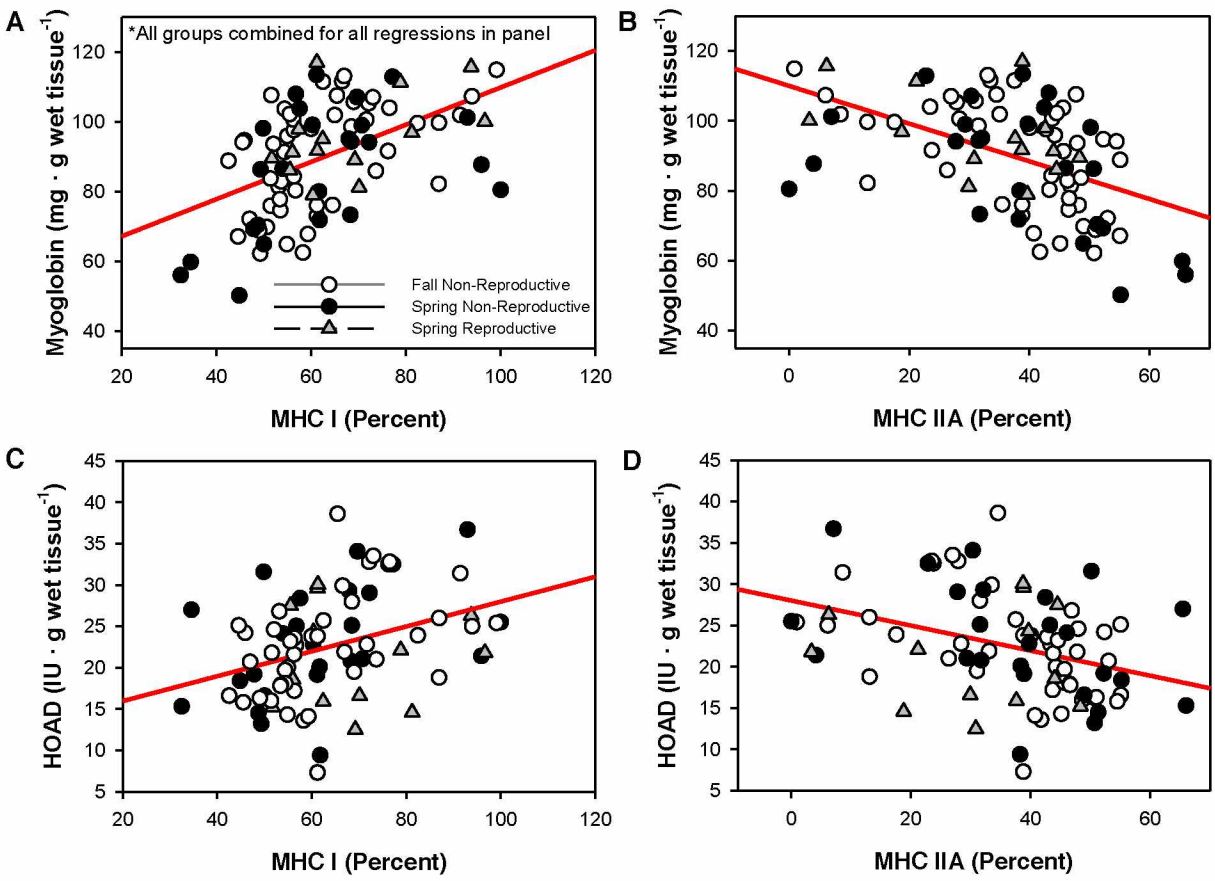

Figure 4.3. Linear regressions showing the relationship between muscle biochemical and structural properties. Muscle (**A-B**) myoglobin and (**C-D**) *β*–hydroxyacyl CoA dehydrogenase (HOAD) activities were significantly correlated with MHC I and IIA composition in the *Longissimus dorsi* swimming muscle. *Single, thick solid red line* = no effect of reproductive group in regression analysis, so trend shows all animals combined.

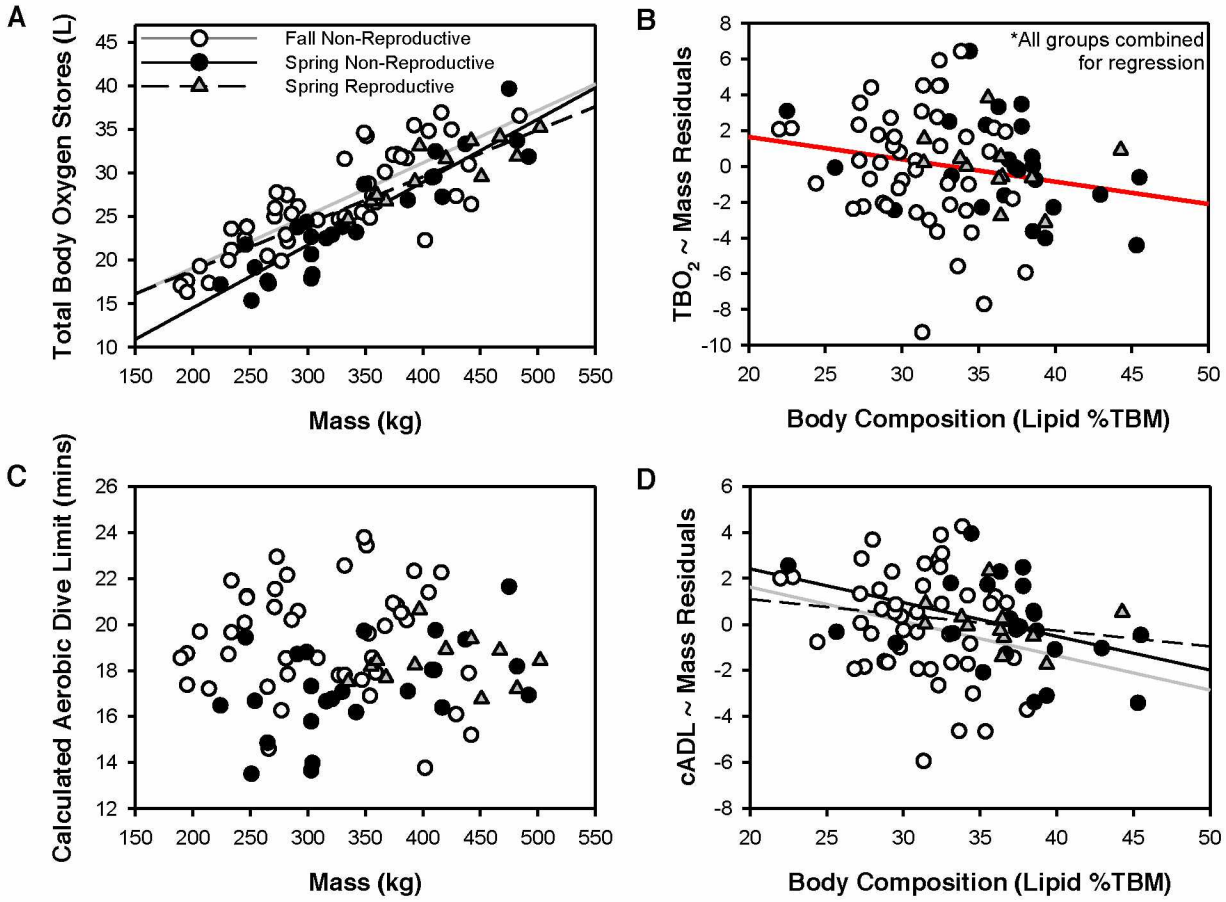

Figure 4.4. The relationship between total body oxygen (TBO_2) stores and the calculated aerobic dive limit (cADL) with Weddell seal body mass and composition. TBO_2 stores were positively correlated with total body mass and the relationship differed by reproductive status (**A**; Fall Non-reproductive: *solid grey line*, Spring Non-reproductive: *solid black line*, Spring Reproductive: *dashed black line*). Body composition accounted for significant variation in the model (**B**; All reproductive groups combined*: Single, thick solid red line*). Conversely, the calculated aerobic dive limit (cADL) did not exhibit a significant relationship with body mass (**C**). Residuals were negatively correlated with body composition, with a reproductive effect (**D**).

Table 4.1. Mean ± SE Weddell seal straight length, total body mass, lean mass, and lipid (as %TBM) among reproductive groups. Sample sizes are shown in parentheses, and *different letters* indicate significant differences between reproductive groups. Note that animal straight length was included as a covariate for total body and lean mass comparisons, and that there were yearly variations in animal mass and lipid stores (*see Shero et al. 2014; 2015*).

	Fall	*Spring*	
Body Condition Parameter	Non-Reproductive	Non-Reproductive	Reproductive
Straight Length (cm)	234.1 ± 2.40 (*53*)[a]	230.5 ± 3.37 (*28*)[a]	250.1 ± 2.81 (*16*)[b]
Mass (kg)	321.6 ± 10.10 (*53*)[a]	335.6 ± 14.30 (*28*)[b]	413.7 ± 13.31 (*16*)[b]
Lean Mass (kg)	221.0 ± 6.73 (*52*)	214.9 ± 9.79 (*27*)	266.5 ± 8.85 (*15*)
Body Composition (%Lipid)	30.88 ± 0.49 (*52*)[a]	36.08 ± 0.99 (*27*)[b]	36.30 ± 0.84 (*15*)[b]

Table 4.2. Mean ± SE blood chemistry parameters with sample sizes shown in parentheses. The relationship of parameters with LMM factors (total body mass; TBM and body composition) are noted: neg. = negative correlation, and ≈ = no significant effect. *Different letters* indicate significant differences among reproductive groups. Plasma and blood volumes are shown scaled to total and lean body mass (TBM and LBM), respectively, and so TBM and body composition were not included in LMM models.

	Mixed Model Relationships		*Fall*	*Spring*	
Hematology Parameter	TBM	Body Composition	Non-Reproductive	Non-Reproductive	Reproductive
HCT (%)	neg.	≈	62.1 ± 0.63 (*53*)^{a}	62.1 ± 0.94 (*31*)^{a}	56.2 ± 1.61 (*17*)^{b}
Hb ($g \cdot dL^{-1}$)	neg.	≈	25.2 ± 0.38 (*53*)	24.6 ± 0.31 (*31*)	23.6 ± 0.74 (*17*)
MCHC (%)	neg.	≈	40.3 ± 0.32 (*52*)	39.8 ± 0.54 (*31*)	39.9 ± 0.66 (*15*)
RBC ($10^{6} \cdot \mu L^{-1}$)	≈	≈	3.88 ± 0.06 (*53*)^{a}	3.82 ± 0.08 (*18*)^{ab}	3.63 ± 0.17 (*12*)^{b}
PV (%TBM)			6.40 ± 0.09 (*53*)^{a}	5.71 ± 0.14 (*28*)^{b}	6.72 ± 0.16 (*16*)^{a}
PV (%LBM)			9.26 ± 0.13 (*52*)^{a}	8.94 ± 0.17 (*27*)^{a}	10.44 ± 0.19 (*15*)^{b}
BV (%TBM)			17.0 ± 0.28 (*53*)^{a}	15.4 ± 0.45 (*28*)^{b}	15.3 ± 0.44 (*16*)^{b}
BV (%LBM)			24.7 ± 0.42 (*52*)	24.0 ± 0.62 (*27*)	23.5 ± 0.55 (*15*)

Table 4.3. Mean ± SE muscle myoglobin (Mb), enzyme activities, and myosin heavy chain composition in the primary locomotor muscle (*longissimus dorsi*) with sample sizes shown in parentheses. The relationships of parameters with LMM factors (total body mass; TBM and body composition) are noted: pos. = positive correlation, neg. = negative correlation, and ≈ = no significant effect. *Different letters* indicate significant differences among reproductive groups. *Asterisk*s indicate tests were run using Kruskal-Wallis tests.

	Mixed Model Relationships		*Fall*	*Spring*	
Muscle Biochemistry Parameter	TBM	Body Composition	Non-Reproductive	Non-Reproductive	Reproductive
Mb (mg·g wet tissue^{-1})	≈	≈	90.4 ± 2.14 (*50*)	86.0 ± 3.52 (*26*)	95.8 ± 3.16 (*14*)
CS (IU·g wet tissue^{-1})	≈	≈	14.9 ± 0.53 (*47*)	14.1 ± 0.68 (*26*)	13.3 ± 1.27 (*14*)
HOAD (IU·g wet tissue^{-1})	≈	≈	23.3 ± 1.04 (*47*)	23.1 ± 1.33 (*26*)	22.9 ± 2.36 (*14*)
LDH (IU·g wet tissue^{-1})	pos.	≈	505.8 ± 16.2 (*47*)^{a}	666.3 ± 24.8 (*26*)^{b}	592.8 ± 29.0 (*14*)^{ab}
CS:HOAD	≈	neg.	0.67 ± 0.03 (*47*)	0.64 ± 0.03 (*26*)	0.60 ± 0.04 (*14*)
LDH:CS	pos.	≈	36.7 ± 2.15 (*47*)^{a}	50.1 ± 2.98 (*26*)^{b}	49.9 ± 5.23 (*14*)^{ab}
MHC I (%)*	≈	≈	61.8 ± 1.9 (*51*)	62.9 ± 3.4 (*27*)	68.3 ± 3.8 (*14*)
MHC IIA (%)*	≈	≈	38.1 ± 1.9 (*51*)	37.0 ± 3.3 (*27*)	31.7 ± 3.8 (*14*)

Table 4.4. Mean ± SE blood, muscle, total body oxygen stores, diving metabolic rate scaled to total and lean body mass (TBM and LBM), respectively, and the calculated aerobic dive limit, with sample sizes shown in parentheses. For all parameters scaled to TBM or LBM, mass and body composition were not included in LMM models. For the cADL, the relationships with LMM factors (total body mass; TBM and body composition) are noted: neg. = negative correlation, and ≈ = no significant effect. *Different letters* indicate significant differences among reproductive groups.

	Mixed Model Relationships		*Fall*	*Spring*	
Oxygen Store Parameter	TBM	Body Composition	Non-Reproductive	Non-Reproductive	Reproductive
Blood O_2 (mL·kg TBM^{-1})			45.4 ± 1.25 (*51*)^{a}	40.1 ± 1.55 (*28*)^{b}	38.1 ± 1.53 (*15*)^{b}
Blood O_2 (mL·kg LBM^{-1})			65.7 ± 1.92 (*50*)	62.4 ± 2.32 (*27*)	58.4 ± 2.14 (*14*)
Muscle O_2 (mL·kg TBM^{-1})			31.8 ± 0.85 (*49*)^{a}	27.7 ± 1.22 (*24*)^{b}	31.5 ± 1.23 (*12*)^{b}
Muscle O_2 (mL·kg LBM^{-1})			45.9 ± 1.10 (*49*)	43.7 ± 1.89 (*24*)	49.4 ± 1.82 (*12*)
TBO_2 (mL·kg TBM^{-1})			83.4 ± 1.55 (*49*)^{a}	72.5 ± 1.59 (*24*)^{b}	73.5 ± 1.37 (*12*)^{b}
TBO_2 (mL·kg LBM^{-1})			120.8 ± 2.24 (*49*)	114.5 ± 2.23 (*24*)	115.5 ± 2.62 (*12*)
DMR (mL O_2·kg TBM^{-1}·min^{-1})			4.30 ± 0.04 (*49*)^{a}	4.22 ± 0.05 (*24*)^{ab}	4.01 ± 0.04 (*12*)^{b}
DMR (mL O_2·kg LBM^{-1}·min^{-1})			6.23 ± 0.06 (*49*)^{a}	6.69 ± 0.12 (*24*)^{b}	6.30 ± 0.12 (*12*)^{ab}
cADL (min)	≈	neg.	19.4 ± 0.33 (*49*)^{a}	17.2 ± 0.42 (*24*)^{b}	18.4 ± 0.30 (*12*)^{ab}

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Chapter 5. Temporal Changes in Weddell Seal Dive Behavior Over Winter: Are Females Increasing Foraging Efforts to Support Gestation?[1]

5.1 Abstract

For Weddell seals (*Leptonychotes weddellii*), the overwinter foraging period (Feb-Sept) is likely critical for females to regain physiological condition prior to pupping the next year (Oct-Nov). We deployed 53 satellite tags on post-molt female Weddell seals in the Ross Sea, and 22 females returned to the area the following year (13 females gave birth; 9 skipped reproduction). Regardless of their reproductive status, females gained similar proportions of mass and lipid stores (*P*'s<0.001) overwinter, and mass-specific O_2 stores and calculated aerobic dive limits (cADL) were maintained across the year. Body size was positively correlated with dive duration and the proportion of dives exceeding the cADL at the onset of winter foraging. Females that returned the following year and gave birth began the post-molt foraging period with significantly longer and deeper dives, as compared to non-reproductive seals. Mean dive duration, indices of foraging efficiency, and the mean proportion of dives exceeding the cADL increased just following the molt, and again prior to the next breeding season. Mid-to-late winter, reproductive females spent a significantly greater proportion of the day diving, and exceeded their cADL more frequently than females that returned without a pup. Analysis of dive bouts revealed that non-reproductive Weddell seals made an average of 2-3 shorter bouts ($BOUT_{short}$; 7.43 ± 0.83 hr) separated by 10-22 minute surface rest periods each day. These shorter bouts were nested within one long daily foraging period ($BOUT_{long}$; 16.3 ± 1.45 hr). In contrast, reproductive females had significantly longer $BOUT_{short}$ durations (1-2 per day; 11.3 ± 1.18 hr) and daily foraging efforts ($BOUT_{long}$; 17.7 ± 2.06 hr), indicating that they required less frequent rest periods. Four main dive types and bout types were identified by cluster analysis, and dive and bout shapes more suggestive of benthic foraging increased through the winter. This study is the first to identify differences in dive efforts by animal reproductive status across gestation, and indicates that successful reproduction is associated with greater foraging effort.

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[1] Shero, M.R., K.T. Goetz, D.P. Costa, and J.M. Burns. 2015. Temporal changes in Weddell seal dive behavior over winter: Are females increasing foraging efforts to support gestation?. Prepared for submission to Marine Ecology Progress Series.

5.2 Introduction

The diving capabilities of central-place foragers, such as marine mammals, are constrained by the magnitude and management of endogenous oxygen (O_2) stores while animals are underwater (Hochachka & Storey 1975; Butler & Jones 1997; Kooyman & Ponganis 1998). Total body oxygen (TBO_2) stores and the rate at which they are consumed are frequently used to calculate the aerobic dive limit (cADL). The cADL provides an estimated threshold duration beyond which blood lactate starts to accumulate above baseline levels (Kooyman et al. 1980; 1983; Ponganis et al. 1993; Burns & Castellini 1996; Costa et al. 2001). Lactate production during anaerobic glycolysis allows animals to extend dive durations. However, if an animal exceeds the aerobic window, more time must typically be spent during the surface recuperation period following a dive in order to metabolize lactic acid generated (Kooyman et al. 1980; Castellini et al. 1988; Fedak & Thompson 1993). The concept of the ADL remains central to the marine mammal field, 35 years later, because the vast majority of dives remain within this threshold (Kooyman et al. 1980; Thompson & Fedak 2001). However, it may be energetically beneficial to animals to exceed their ADL in order to exploit rich prey patches if acquisition of these resources outweighs the costs of longer post-dive recovery times (Houston & Carbone 1992). Therefore, an optimal dive time balances the need to acquire ephemeral prey resources, with depleting O_2 stores and the additional costs of exceeding the ADL (Kramer 1988; Houston & Carbone 1992; Thompson & Fedak 2001).

The "optimal" dive duration likely changes across the year in response to prey abundance and distribution and plasticity in the animal's aerobic capacity. There are numerous documented cases in which marine mammal dive durations far exceed estimated aerobic capacities on a routine basis (Castellini et al. 1988; Hindell et al. 1992; Boyd & Croxall 1996; Schorr et al. 2014). For example, southern elephant seals (*Mirounga leonina*) dive significantly longer during the post-molt foraging period as compared to the post-breeding period, exceeding the cADL more often (Hindell et al. 1992). Additionally, benthic foraging otariid species tend to exceed their cADL more often than pelagic foraging species of similar size (Costa et al. 2001; 2004; Chilvers et al. 2006). Marine mammals also experience drastic changes in activity budgets, with prolonged periods of fasting and dramatic weight loss (Costa et al. 1986; Wheatley et al. 2006; McDonald et al. 2008; Crocker et al. 2014) associated with critical life history events such as

parturition, nursing, and molting (Kooyman 1975; Castellini et al. 1992; Schreer & Testa 1996; Forcada et al. 2012). Changes in body mass and metabolic rates, and any atrophy of O_2-storage proteins due to reduced exercise and hypoxia exposure have the potential to alter cADL values (Hochachka et al. 1998; Hoppeler & Vogt 2001; Halvorsen & Bechesteen 2002; Haddad et al. 2003). The foraging period directly following critical life history events associated with loss of mass and condition would be important for recuperation of energy stores for the next year.

The ability to acquire energy reserves over long foraging periods coinciding with gestation also has the potential to influence reproductive outcomes. Foraging success during the gestation period could impact whether pregnancies are carried to full-term and also how much of the female's body reserves can later be allocated to neonatal growth, potentially impacting pup survival (Proffitt et al. 2007; 2008). However, relatively few studies have identified differences in dive behavior that are associated with reproductive success (Slip et al. 1994). Because female Weddell seals (*Leptonychotes weddellii*) allocate the same amount of energy towards "self-preparation" over the winter foraging period, regardless of whether they produce a pup or not the following year, and the only difference in tissue and energy accretion during this foraging period comes from fetal tissue and growth (i.e., gestating females need to increase energy gains by ~13% relative to non-reproductive seals; Shero et al. 2015; Chapter 3 *this thesis*), any differences in diving efforts over the austral winter may be attributed to gestational costs. Therefore, Weddell seals offer a unique opportunity to assess whether female dive behavior differs in measureable ways between females that successfully give birth following the winter post-molt foraging period, and those that do not.

This study aims to assess changes in Weddell seal diving behavior across the austral winter and to test whether foraging patterns differ between females that return the following year with or without a pup. In particular, we assess whether foraging efforts are elevated just after the annual molt when animals are leanest, and may need to quickly regain body mass. Therefore, we examine traditional metrics of foraging effort and success (dive duration, depth, bottom time at >80% the maximum depth of a dive) using dive recorders and telemetric methods, and develop several new proxies of foraging effort. For example, bouts of successive dives can be characterized to assess physiological dive capacity and this study extends the idea of "bottom" time correlating with foraging effort to also include dive bouts. Exceeding the cADL would also

be an indicator of times of increased foraging effort. Identifying the relationships between physiology and behavior in a top, marine predator would help to elucidate the "buffer" that animals have during environmental perturbations, such that additional aerobic scope would maintain foraging efficiencies.

5.3 Methods

5.3.1 Animal Handling

Fifty-three post-molt adult female Weddell seals were captured on the fast-ice along the McMurdo Sound region, Antarctica in Erebus Bay (~77°S, 165°E) and the Victoria Land coastline (~76°S, 162°E) in January/February (austral fall) 2010-2012. All the post-molt females in this study were assumed to be non-reproductive (i.e., did not give birth earlier in the year). This conclusion is based on the fact that of the females seen in Erebus Bay during the breeding season and resighted during the molt, none of our handled females (n = 25), and <15% of fully molted females in the population overall, had a pup the prior spring (October/November; Burns et al. 2013; Beltran & Burns *unpublished*). Animals were sedated with an initial intramuscular dose of approximately 1.0 $mg \cdot kg^{-1}$ tiletamine/zolazepam HCl. Following a 10 to 15 minute induction period, animals were captured via hoop net and additional intravenous injections of ketamine and diazepam (~0.2 $mg \cdot kg^{-1}$ and 0.012 $mg \cdot kg^{-1}$) were administered approximately every 10 mins, or as necessary, to keep animals sedated while remaining eupneic. At each handling, animals were weighed using a sling, tripod, and scale (MSI-7200-IT Dyna-Link digital dynamometer, capacity 1,000 ± 1.0 kg). Body composition (%lipid) was measured using tritiated water dilution as described in Shero et al. (2014). Blood and muscle O_2 stores were measured to calculate an aerobic dive limit for each animal and muscle lactate dehydrogenase (LDH) activities were measured as an index of anaerobic potential, as described in *Chapter 4 (this thesis*).

All post-molt females were outfitted with Conductivity Temperature Depth-Satellite Relay Dive Loggers (CTD-SRDLs) weighing 600 g from the Sea Mammal Research Unit (University of St. Andrews, St. Andrews, Scotland). Tags were attached to the fur on the animals' heads using 5

minute epoxy (Loctite® or Devcon®). Data were transmitted as compressed dives (Fedak et al. 2002) to CLS ARGOS (Collecte Localisation Satellites, Advanced Research and Global Observation Satellite System). Twenty females were re-captured the following spring (265.7 ± 5.1 days later), but only 18 tags had transmitted dive records. Four more animals were seen (i.e., known pregnancy outcomes) but could not be handled due to logistical constraints. In total, 13 of the females that returned the following year (t+1) gave birth, and nine returning females did not produce a pup. Reproductive females were handled an average of 7.3 ± 1.5 days post-partum. Of recaptured females, only ten were still carrying their tags at the time of the second handling. Instruments were physically recovered from these ten returning animals and contained complete dive records (i.e., no ARGOS transmission loss). Dive data from recovered tags were used wherever possible for the rest of this manuscript; transmitted data were used when recovered data were not available.

5.3.2 Dive Processing

A dive was defined as an underwater event that lasted for 4x the sampling interval / depth resolution of data loggers, or >12 m but < 2000 m in depth and >16 seconds in duration. Only dives with vertical travel speeds < 5 m/s were retained, and additional outliers were detected visually using dive depth versus duration plots and discarded. Activity budgets (%time spent diving, at surface, and hauled-out) and the number of dives made were collected in four, 6-hr intervals each day. Activities were averaged and number of dives summed for the full-day, only when all data were available for the full 24-hr period.

Dive records contained four main inflection points where the largest change in trajectory occurs for each individual dive (Fedak et al. 2002). Bottom time, foraging efficiency, and dive shapes were determined by interpolating the four major inflection points transmitted per dive, into 100 equal mid-depths. Each interpolated point at >80% the maximum depth for a given dive was considered to be "bottom" time. Foraging efficiency was then calculated as:

$$\text{(Eq. 5.1)} \quad \text{Foraging Efficiency (\%)} = \frac{\text{Bottom Time}}{(\text{Dive Duration} + \text{Surface Duration})}$$

Dive shapes were identified by first calculating 10 mean mid-depths per dive, and then using K-means cluster analyses and R^2 and pseudo F statistics to identify the number of unique clusters (Schreer et al. 1998; 2001). Additionally, dive descent and ascent rates were calculated as the meters travelled per second from the initiation of a dive to the first inflection point, and the last inflection point to the termination of a dive, respectively (Biuw et al. 2003). Dive durations were also compared to the cADL, calculated as TBO_2 stores divided by an average diving metabolic rate (DMR) of 1.6 × Kleiber (Kleiber 1975; Williams et al. 2004; Chapter 4 *this thesis*).

5.3.3 Links Between Physiology and Dive Behavior

5.3.3.1 Influence of Animal Size

To evaluate factors that may influence dive behavior at the onset of winter foraging (i.e., first 8 weeks post tag deployment), body mass and body composition were correlated with mean dive metrics for all 53 study animals for which at least 100 dives were available, using linear regression analyses. Metrics included in these analyses were mean dive duration (>3 min due to bimodal distribution), number of dives per day, depth, bottom time, foraging efficiency, %dives exceeding the cADL, and %day spent diving. A separate regression analysis that included reproductive outcome was run for only those 22 females recaptured during the subsequent breeding season to assess whether dive metrics differed between reproductive and non-reproductive females.

5.3.3.2 Overwinter Dive Behavior Between Reproductive Groups

Of the 22 females with known reproductive outcomes the following year, two tags did not transmit sufficient data, and these animals were excluded from analysis across the winter. For the remainder of females, generalized additive mixed models (GAMMs with the "mgcv" package in R v. 0.98.490) were used to determine how dive and surface duration, depth, frequency, shape, bottom time, foraging efficiency, activity budgets, and the proportion of dives exceeding the cADL changed across the year and in response to reproductive status. Julian day was used as a smoother in models, reproductive status in year t+1 was a factor (with and without a reproductive status × Julian day interactive effect), and animal ID was a random effect with a penalized thin-plate regression spline. To provide more detailed temporal comparisons, dive metrics were also

compared between reproductive groups within each month. Models had a gamma distribution with a log link, Gaussian, or (negative) binomial distribution, and were validated to ensure there was not overdispersion or heterogeneity of residuals. The best-fit model was identified using Akaike Information Criterion tests corrected for small sample size (AICc) in the R "MuMIn" package (Zuur et al. 2014). A linear interpolation was used to transition from the TBO_2 and total body mass measured in fall to values measured in spring for the same individual when measured in both seasons (Non-Reproductive: n = 4, Reproductive: n = 8; other animals were excluded due to incomplete handlings and logistical constraints). For each day during the tag deployment, estimated TBO_2 and body mass (used to estimate DMR) from linear interpolations were used to calculate a cADL, and each dive was ranked as either being greater or less than the cADL before a GAMM with binomial distribution was used to assess the probability of exceeding the cADL across the winter.

5.3.4 Characterization of Dive Bouts Over Winter

For the 10 animals for which the entire overwinter dive records were obtained from recovered tags, dives were grouped into bouts to assess organization of larger foraging efforts. Because SMRU CTD-SRDL tags record surface time and haul-out time separately, the time difference was taken between the end of one dive and the start of the next to calculate a full, post-dive recuperation period. This could only be done for complete records as missing dives would concatenate surface intervals and artificially shorten bouts. A two-process non-linear least squares model assuming a Poisson distribution was fit to log frequency plots of post-dive surface intervals for each animal to divide surface gaps into short (within bout) and long processes (between bouts; Slater & Lester 1982; Feldkamp et al. 1989; Sibly et al. 1990; Berdoy 1993). This method yielded bout-ending criteria (BEC) that were longer than expected for pinnipeds (Boyd et al. 1991; Harcourt et al. 2002), and visual inspection through these dive records suggested that the two-process model overestimated bout lengths. To test this hypothesis, three process models were fit to post-dive surface intervals, identifying BEC1 (fast to slow process for dives within small bouts; $BOUT_{short}$) and BEC2 (slow to slower process for small bouts nested within larger bouts; $BOUT_{long}$). All models were fit using the "diveMove" package in R, following methods outlined in Sibly et al. (1990) and Berdoy (1993), and two- versus three-process model fit was compared using AICc and ANOVA F-tests. A $BOUT_{short}$ and $BOUT_{long}$

was then considered to be >5 dives made within BEC1 and BEC2, respectively, and bout characteristics (i.e., duration, number of dives, number of $BOUT_{short}$ within $BOUT_{long}$) were assessed across the year and between reproductive classes using GAMM models.

In an attempt to characterize bout types based on the frequency and depth of dives within them, bout shapes were also identified. Bout shapes were determined in a similar fashion to shapes of individual dives; however, the maximum depth of each dive within the pre-defined bout was used as the initial points defining the bout 'shape' and 100 midpoints were interpolated to create shape clusters in a similar fashion to analyses on individual dives. Finally, bout efficiency was calculated as:

$$\text{(Eq. 5.2)} \qquad \text{Bout Efficiency}(\%) = \frac{\%\text{Dives reaching >80\% Max Dive Depth}}{(\text{Bout Duration} + \text{Post Bout Surface Interval}).}$$

The proportion of total dives that occurred within bouts was determined, and the last dive in a bout was compared to all dives within the bout using GAMM analyses to determine whether bouts ended after exceeding physiological capacities (i.e., particularly long duration or deep dives).

5.4 Results

5.4.1 Physiological Links with Start of Winter Foraging

During the first 8 weeks of winter foraging, larger animals made significantly fewer (Fig. 5.1A; $F_{1,44} = 11.4$, $R^2 = 0.205$, $P = 0.002$), but longer (Fig. 5.1B; $F_{1,44} = 54.3$, $R^2 = 0.552$, $P < 0.001$) dives as compared to smaller Weddell seals. However, because mean dive depth did not differ (Fig. 5.1C), larger animals were able to spend significantly more time at the bottom of dives (Fig. 5.1D; $F_{1,42} = 11.0$, $R^2 = 0.207$, $P = 0.002$), and reproductive seals had longer bottom times than expected for their size ($P = 0.040$). Yet, this increase was proportional and larger seals did not spend a greater proportion of their time at the bottom of dives or have greater foraging efficiencies (Fig. 5.1E-F). However, foraging efficiency was strongly positively correlated with dive depth (Fig. 5.1G; $F_{1,42} = 14.2$, $R^2 = 0.253$, $P < 0.001$). Surprisingly, the largest seals most

frequently exceeded their cADLs ($F_{1,40} = 57.7$, $R^2 = 0.569$, $P < 0.001$), although this behavior did not appear to compromise their total time foraging, as all seals spent a similar amount of time diving each day (Fig. 1H-I). Animal body condition (lipid stores) and anaerobic LDH activities did not significantly improve model fit. Similarly, reproductive status was not a significant factor in other regressions between mass and dive behaviors in the 8 week period following the molt.

5.4.2 Overwinter Foraging Between Reproductive Groups

Despite the fact that O_2 stores did not change seasonally (Chapter 4, *this thesis;* Table 5.1), dive behavior differed markedly across the year (all behaviors listed below ~ Julian day $P < 0.001$). For example, all seals increased dive durations and depth (Fig. 5.2A-B) mid-winter from ~May until the next breeding period in October. During those months when animals made longer and deeper dives, they also made fewer dives per day. Dive frequencies and daily dive activity increased directly post-molt from January until April (Fig. 5.2C-D). There was a sharp decline just prior to the breeding season, as dives became longer and deeper.

Further, gestating seals tended to behave in ways suggestive of increased dive efforts relative to non-reproductive seals across the austral winter. Females that returned the next year with a pup made slightly longer dives across the austral winter (Fig. 5.2A), and dive durations were significantly greater immediately post-molt (January-February) and mid-winter (August). Reproductive females also made deeper dives at the start of the overwinter foraging period, and this pattern persisted throughout the winter (Fig. 5.2B; $P = 0.048$). Reproductive females spent a slightly larger proportion of the day diving as compared to non-reproductive seals across the entire winter (Fig. 5.2D), but the difference was only significant during mid-winter (June-August).

Bottom time (minutes and percent), foraging efficiencies, and the proportion of dives exceeding the cADL were highest just following the molt (January-February) and at the end of the winter foraging period (September-October) (Fig. 5.3A-D). Further examination revealed that reproductive females had longer bottom times and higher foraging efficiencies, at the start of the winter foraging period, while non-reproductive seals showed a sharp increase in bottom time and foraging efficiency in the spring (Fig. 5.3A-C). There was an increasing trend in the proportion of dives exceeding the cADL across the winter foraging period. All animals started exceeding

their cADL more often in the few weeks marking the start of winter foraging (post-molt), and exceeded aerobic capacities most often just prior to the breeding season the next year. In spring (September-October) reproductive females exceeded the cADL significantly more often than non-reproductive seals (Fig. 5.3D). Four dive shapes were identified, with the majority of dives being long and deep square-shape dives (Table 5.2).

5.4.3 Characterization of Dive Bouts

For all animals with complete dive records, three process models provided significantly better fit to log frequency post-dive surface interval plots (Table 5.3), as compared with two process models. The vast majority of dives were performed in bouts consisting of >5 successive dives ($BOUT_{short}$: 93.8 ± 1.2%; an additional 5.4% of dives within $BOUT_{long}$). Only 0.8% of dives were not included in $BOUT_{short}$ or longer trips to sea ($BOUT_{long}$). The bout-ending criteria (post-dive surface intervals between processes; BEC1 and BEC2) did not differ between reproductive groups (BEC1: $t_{8.0} = -0.1$, $P = 0.966$; BEC2: $t_{7.7} = -1.2$, $P = 0.275$). The number of dives per $BOUT_{short}$ and $BOUT_{long}$ were slightly, but not significantly, greater in reproductive females during the winter overall (Fig. 5.4A-B), but reproductive seals made significantly more dives in $BOUT_{short}$ during April and June. Reproductive females did have significantly longer $BOUT_{short}$ durations than non-reproductive females (P = 0.016), but non-reproductive females made significantly more $BOUT_{short}$ within $BOUT_{long}$ as compared to reproductive females ($P < 0.001$). Both non-reproductive and reproductive females had similar $BOUT_{long}$ durations (Fig. 5.4C-E). Surface intervals between $BOUT_{long}$'s exhibited an inverse relationship with bout duration, and non-reproductive females tended to rest for slightly longer periods between long bouts (Fig. 5.4F). The last dives in $BOUT_{short}$ were significantly shorter in duration (Fig. 5.5; $P < 0.001$) and also shallower ($P < 0.001$), as compared to all dives in the bout.

Cluster analyses were only conducted on $BOUT_{short}$ as these comprised >90% of dives, and revealed four main $BOUT_{short}$ shapes, with relatively even distributions of left skewed-"V", deep-square, shallow-square, and right skewed-"V" shaped bouts (Table 5.4). Deep-square $BOUT_{short}$ tended to be comprised of fewer dives that were of longer duration and exceeded the cADL more often, reaching greater mean depths, and having greater within-bout dive:surface ratios (Table 5.4). Animals tended to take multiple successive square-shape bouts, as a square-shape bout was

most likely to be followed by another square-shape "foraging" bout. The frequency of deep-square $BOUT_{short}$ exhibited a marked increase from mid-winter until the next breeding season (Fig. 5.6). Consequently, the frequency of all other $BOUT_{short}$ types declined just prior to the subsequent spring. None of the bout type frequencies showed overall overwinter differences by reproductive group.

5.5 Discussion

This study shows that there is significant variation in dive behavior across the post-molt foraging period in female Weddell seals, due to both individual and seasonal factors. The numerous differences in dive capabilities and foraging effort that were attributable to animal size and reproductive class suggests that physiological condition at the end of the annual molt strongly influences foraging strategies at the onset of winter. Further, persistent differences in diving behaviors between reproductive and non-reproductive females strongly support the idea that Weddell seal females meet the additional energetic costs of supporting fetal growth by increasing dive durations and the proportion of each day spent diving.

Body mass just following the annual molt strongly influenced many aspects of dive behavior as has been observed in other pinniped species (Hassrick et al. 2010; McIntyre et al. 2010). In female Weddell seals, mass and body composition (lipid stores) are lowest at the end of the molting period, and significantly increased across the austral winter (Shero et al. 2015). Because TBO_2 stores remain similar across the year (Chapter 4, *this thesis*), limitations on dive duration at the end of the annual molt are more likely due to reduced body mass, which increases mass-specific diving metabolic rates ($Mass^{0.75}$; Kleiber 1947; 1975; Kooyman 1989). Within the Erebus Bay area at the start of tag deployment, local animal dive depths may have been constrained by shallow bathymetry surrounding Ross Island (Testa 1994; Eakins & Sharman 2010), or alternatively, animals may have been targeting prey that inhabit specific layers of the water column. In both cases, diving longer would only allow individuals to forage at the benthos longer or at the depth layer where preferred pelagic prey were found ("bottom", >80% maximum dive depth), not travel to deeper depths.

Larger animals also exceeded their cADL more frequently than smaller individuals during the eight-week period post-tag deployment (post-molt), suggesting that average diving metabolic rates may overestimate true values of larger individuals. This would result in larger seals having longer true aerobic dive limits than were calculated. Alternatively, larger animals may have indeed been exceeding aerobic capacities more often, and adopted different foraging strategies, such as has been observed between sexes in pinniped species with size-dimorphism (Le Boeuf et al. 1993; Beck et al. 2003; Page et al. 2005). Larger animals may have exploited rich prey patches past their aerobic threshold and simply taken fewer, longer dives throughout the day than smaller seals. Ultimately, these longer dives did not require animals to take such long surface recuperation periods that daily foraging activities were reduced. Whether animals opted to make few, long dives or many, short dives, the total proportion of time spent diving each day during the period directly post-molt was similar across the entire animal size range.

In addition to fueling self-maintenance costs, post-molt foraging must provide enough energy to fuel gestational costs. The proportion of females returning in year t+1 that gave birth in this study was similar to that of the overall population (Hadley et al. 2006; 2007; Proffitt et al. 2007; Chambert et al. 2013). Because females that successfully produce a pup in year t+1, and those that do not, allocate the same amount of energy reserves towards "self" across the austral winter (Shero et al. 2015), differences in dive behavior between these groups likely reflect the additional foraging required to maintain a fetus (Brody 1945). Despite having similar TBO_2 storage capacities as non-reproductive seals (Table 5.1, Chapter 4, *this thesis*), gestating females increased foraging efforts primarily by increasing their dive depths and durations, and this drives an increase in the relative proportion of each day spent diving and dives exceeding the cADL. This was particularly true in late winter, during the last trimester of pregnancy when energetic costs of gestation reach a maximum (Brody 1945). In addition, reproductive females spend an average of 1.09 more hours (8.9% more time) each day diving during most of the winter. Assuming that more time spent diving correlates with higher prey intake, these patterns are similar to the 10-15% increased food intake observed during pregnancy in humans (Rosso 1987), and may reflect a response to endocrine factors that regulate appetite during pregnancy (Grattan et al. 2007; Hirschberg 2012). The increase in dive time across gestation also closely matches the estimated 13.4% increase in energetic demand during pregnancy from these same individual animals (Shero et al. 2015; Chapter 3, *this thesis*).

Many indicators of foraging effort (dive duration, bottom time, foraging efficiency) were greater in gestating seals directly following the molt as compared to females that failed to produce a pup the next year, suggesting that Jan/Feb may be a critical time for females to maintain early pregnancy and prevent embryo loss (Pitcher et al. 1998). This also suggests that the period directly post-molt is critical for animals to regain mass and condition (lipid) lost during the breeding season before the onset of winter (Carlini et al. 1999; Beck et al. 2003; McDonald et al. 2008; Robinson et al. 2012). However, because there is also the possibility that females were pregnant at the time of handling, and non-reproductive females simply lost the fetus later during the austral winter, this study is unable to distinguish whether (A) only the gestating animals increased dive durations or (B) all animals were pregnant in Jan/Feb but animals that did not increase diving efforts did not regain sufficient condition to maintain a pregnancy (Boyd 1984; Pitcher et al. 1998).

Differences in dive duration between reproductive and non-reproductive females may be driven by pregnancy-induced changes in metabolic rate. Early pregnancy may be associated with hypometabolism and lengthening of the aerobic window (King 2000; Sparling et al. 2006), while late pregnancy may lead to higher metabolic rates (Prentice et al. 1989), thus limiting dive durations. That lengthening dive durations is important to pregnant females is also suggested by an increase in the proportion of dives exceeding the cADL overwinter, as compared to non-reproductive females. However, if pregnancy is associated with decreased diving metabolic rates in Weddell seals, the cADL of reproductive females would have been slightly underestimated in this study. Mid-winter (~June-August), reproductive females show the greatest difference in dive duration and activities from non-reproductive seals. This time coincides with the period that pregnancy becomes most energetically-costly at approximately five months (Brody 1945). Towards the end of the winter foraging period, reproductive female dive durations reached a plateau, potentially reflecting increased late-gestation metabolic rates (Prentice et al. 1989), while non-reproductive females increased dive times.

Another marked increase in foraging effort occurred in the austral spring (August-October) when all females increased dive depths and square-shape foraging bouts. Yet, only the non-reproductive females increased dive durations, bottom time, foraging efficiency, and square-shape individual dives just prior to the breeding season. This suggests that late spring foraging

may be particularly important to provide skip-breeding females the opportunity to gain last minute energy reserves before the next breeding season and molting periods. In contrast, in September-October reproductive females show declines in proxies of foraging effort, when they are likely to be returning to the breeding colonies and staying hauled-out on the fast-ice as they begin the pupping season. Because females did not have higher O_2 storage capacities at the time of the breeding season the following year, our study shows that diving longer over the winter leads to an increase in the proportion of dives exceeding the cADL, reaching a maximum during the next spring. Longer dive durations exceeding the cADL throughout the post-molt foraging period have likewise been observed in other pinniped species (Hindell et al. 1992; Chilvers et al. 2006). Further assessments of where Weddell seals operate relative to their ADL would benefit from additional studies of seasonal changes in DMR, as the increases in dive duration from May-October could also have been caused by lower metabolic rates and lengthening of the true ADL. In contrast to aerobic capacities, these individuals exhibited increased anaerobic potential, with muscle lactate dehydrogenase (LDH) activities used as proxy (Table 5.1; Chapter 4 *this thesis*). Longer dive durations at the end of winter may have increased production of LDH protein or increased enzyme activity (Semenza et al. 1994; Mujika & Padilla 2001). Either would help to support exceeding the cADL, and also facilitate faster clearance of lactate during the post-dive recuperation period (Thompson & Fedak 2001; Davis et al. 2004). Faster breakdown of anaerobic byproducts would decrease surface time and maintain high foraging efficiencies, as was also observed in non-reproductive females in the spring.

Another possible reason for the seasonal shifts in diving behavior may be due to changes in habitat utilization and overwinter seal movements, prey distribution, and prey capture success. Over winter, Weddell seals travel from Erebus Bay towards regions where more productive circumpolar deep water is advected onto the Ross Ice Shelf up the canyons between the Pennell and Mawson Banks (Goetz *pers. comm.*). In the spring when the Ross Sea transitions from polar night to high-light conditions, prey shifts in the water column may force Weddell seals to reach greater depths and exceed the cADL to attain prey (Croxall et al. 1985; McConnell et al. 1992). Animals may also be preferentially diving to attain larger prey items at this time, as Antarctic silverfish (*Pleuragramma antarcticum*) comprise the largest component of Weddell seal diet, and larger specimens reside at >200 m depths (Hubold 1984; Hubold & Ekau 1985; Burns et al. 1998; Goetz *pers. comm.*). That reproductive Weddell seals consistently dive to greater depths

throughout the austral winter suggests that they may have been targeting more energy-dense prey items to fuel the additional costs of gestation. There is evidence that Weddell seals have higher prey-capture success rates during daylight hours (Fuiman et al. 2014), which in the study area occur only between September-April, as the sun does not rise from May-August. Therefore, these greater energetic gains may outweigh the additional costs of diving longer, deeper, and exceeding the cADL during particular times of the year.

This study is the first to identify a three-process model fit to pinniped dive behavior performed in bouts. The three-process model identified both the daily activity pattern of Weddell seals ($BOUT_{long}$) followed by one long period when animals are likely hauled-out on the ice substrate. When at sea, Weddell seals structured their behavior into shorter bouts ($BOUT_{short}$). The last dive in bouts was typically short and shallow relative to the rest of the bout. Short bouts may end once CO_2 and lactate build-up occurs after multiple successive dives, and animals may use these inspiratory cues to return to the surface and rest (Stephenson 1991) before beginning the next $BOUT_{short}$ (within 50-111 mins). Alternatively, the end of a bout may have marked the capture of large prey, and BEC1 post-dive surface intervals may have been spent processing these resources, as opposed to being a physiological recuperation constraint. Indeed, Weddell seal encounters with toothfish (~70-90 kg; *Dissostichus mawsoni*) have been shown to occur relatively early in dives (Fuiman et al. 2007), and successful prey capture results in the seals bringing large Antarctic toothfish to the surface at dive holes for caching or processing, which involve a lengthy procedure of discarding the head and skin of the fish prior to consumption (Ponganis & Stockard 2007). Non-reproductive females had significantly shorter $BOUT_{short}$ durations, requiring more small bouts nested within $BOUT_{long}$ to achieve equivalent long foraging periods. Therefore, it appears that non-reproductive seals required longer breaks within their daily foraging activities.

In combination, the differences in dive and bout patterns seen in animals of different size and reproductive class may reflect different foraging niches among individuals with different physiological capacities and energetic demands (Harcourt et al. 2002; Weise & Costa 2007). This study is the first to highlight the different foraging and energetic requirements to bring a fetus to term in a top marine mammal predator. In addition to body mass significantly impacting many dive parameters indicative of successful foraging, reproductive status accounted for

significant variation in dive behavior across the austral winter. That gestating female Weddell seals only exhibit modest energetic gains during the post-molt foraging trip relative to other pinniped species (Shero et al. 2015) despite increases in dive depth, durations, dives exceeding the cADL, the total amount of time spent diving each day, and less rest surface periods during foraging bouts suggests that this species may be operating closer to their physiological limits during gestation. This would make Weddell seal reproductive outcomes particularly vulnerable to environmental perturbations that would alter prey abundance and predictability.

5.6 Acknowledgements

We are grateful for the help of field team members: Linnea Pearson, Dr. Patrick Robinson, and Dr. Luis Hückstädt for sample collection. Group B-009-M led by Drs. Robert Garrott, Jay Rotella, and Thierry Chambert provided information regarding animal reproductive status and provided great assistance in locating study animals. Logistical support was provided by the National Science Foundation (NSF) U.S. Antarctic Program, Raytheon Polar Services, and Lockheed Martin ASC; we thank all the support staff in Christchurch, NZ and McMurdo Station. This research was conducted with support from NSF ANT-0838892 to D.P.C. and ANT-0838937 to J.M.B. This material is based upon work supported by the National Science Foundation Graduate Research Fellowship under Grant No. DGE-1242789 to M.R.S. Any opinion, findings and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation. Animal handling protocols were approved by the University of Alaska Anchorage and University of California Santa Cruz's Institutional Animal Care and Use Committees. Research and sample import to the United States were authorized under the Marine Mammal permit No. 87-1851-04 issued by the Office of Protected Resources, National Marine Fisheries Service. Research activities were also approved through Antarctic Conservation Act permits while at McMurdo Station.

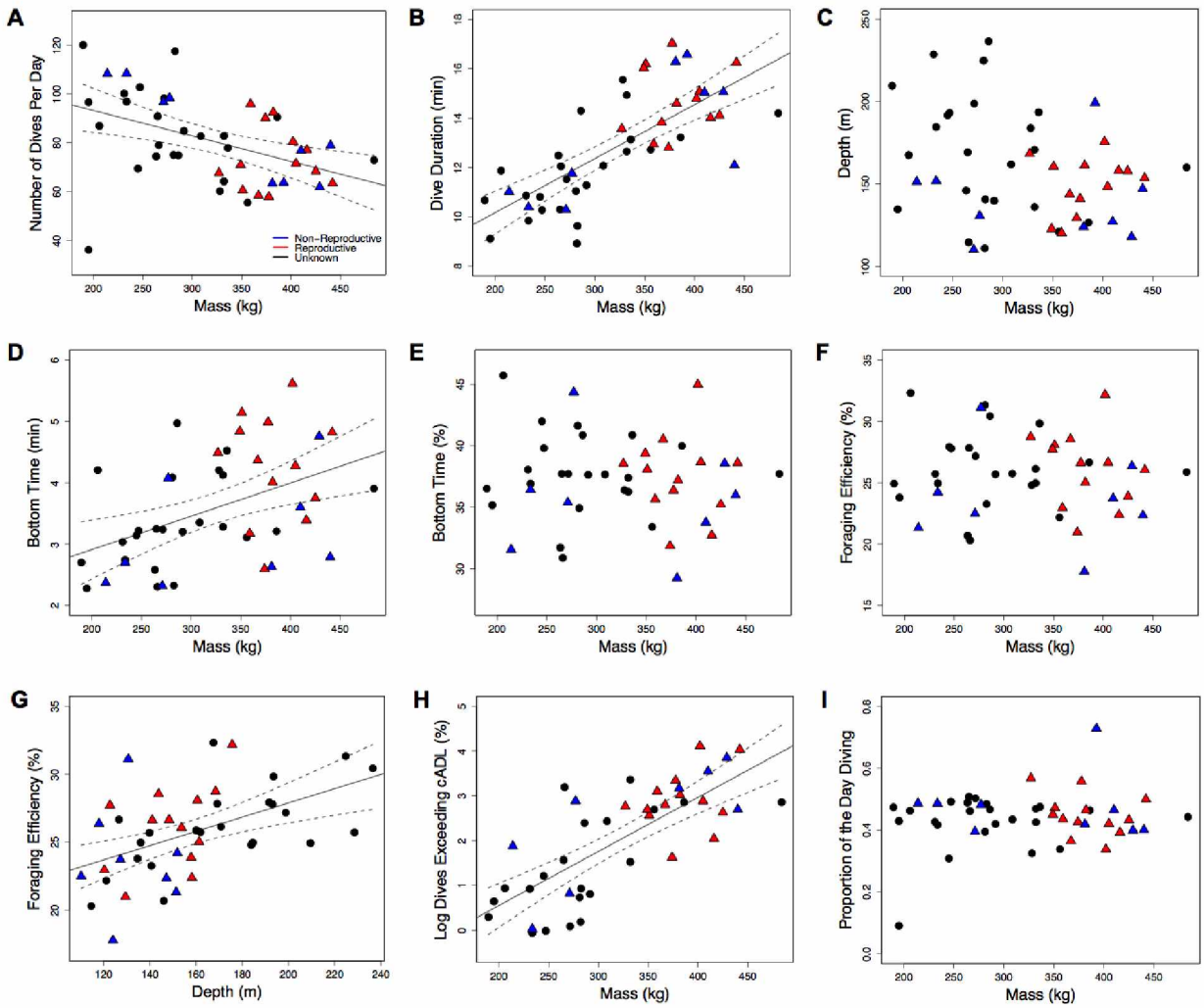


Figure 5.1. Linear regressions showing the relationship between Weddell seal dive (**A-F**) frequency, duration, depth, bottom time (>80% maximum depth per dive), percentage of dives spent at the "bottom", and foraging efficiency with body mass during the first eight weeks post-molt tag deployment, and (**G**) depth was highly correlated to foraging efficiency. (**H**) Larger animals exceeded the cADL more often than smaller seals, but (**I**) all animal size classes spent similar proportions of the day diving. *Black* circles = animals with unknown reproductive histories the following year. *Blue* triangles = Non-reproductive; and *Red* triangles = Reproductive females in year *t*+1.

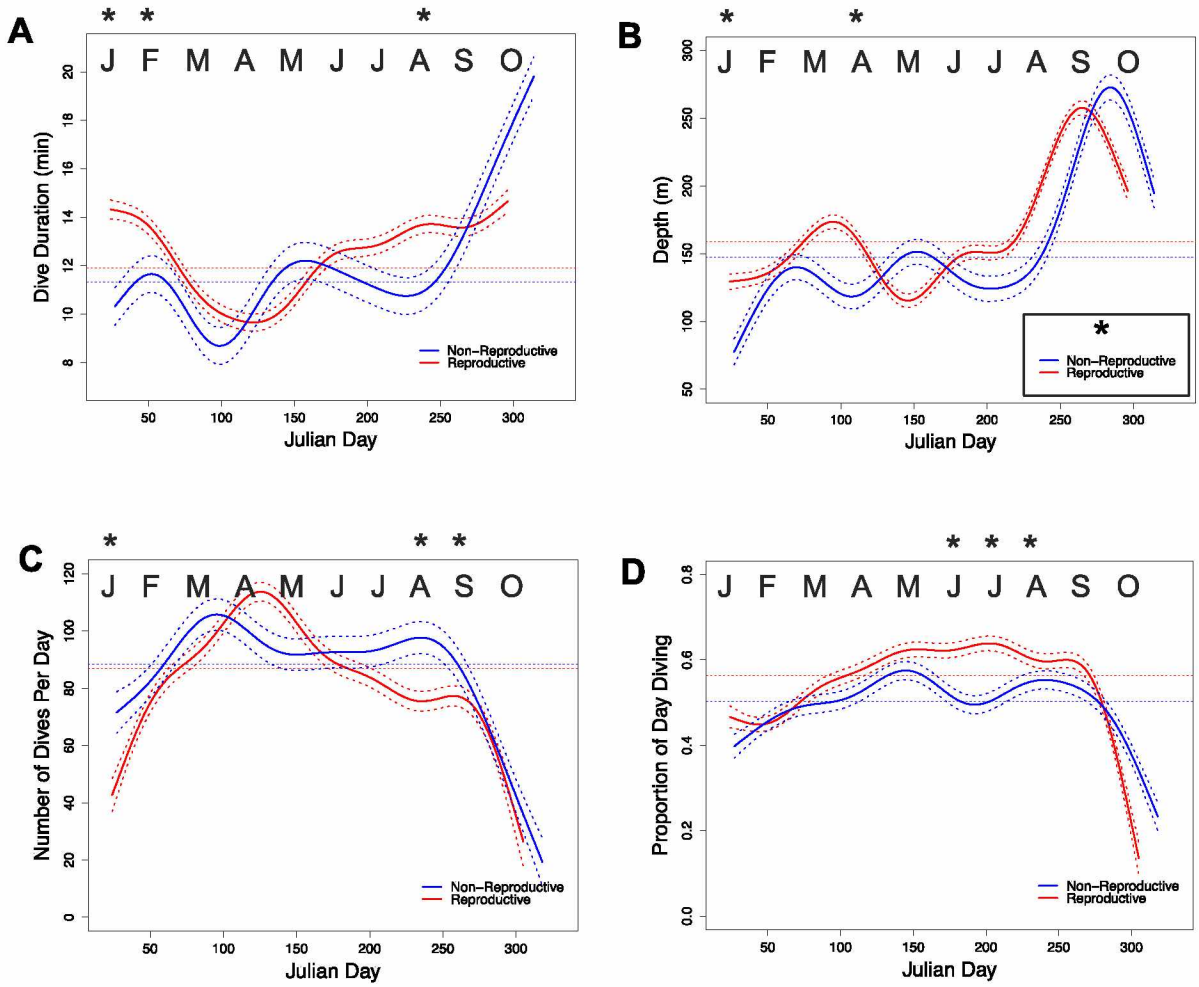


Figure 5.2. Generalized additive mixed models showing trends in dive duration, depth, number of dives made per day, and the proportion of each day spent diving across the year. Julian day had a significant effect on all dive parameters. Reproductive groups are shown as *blue* = Non-Reproductive; *red* = Reproductive. Month is abbreviated at the top of each panel and *Asterisk* = significant differences between reproductive groups for a given month. *Boxed asterisk* = significant reproductive difference across the entire austral winter overall. Horizontal lines represent means across the tag deployment.

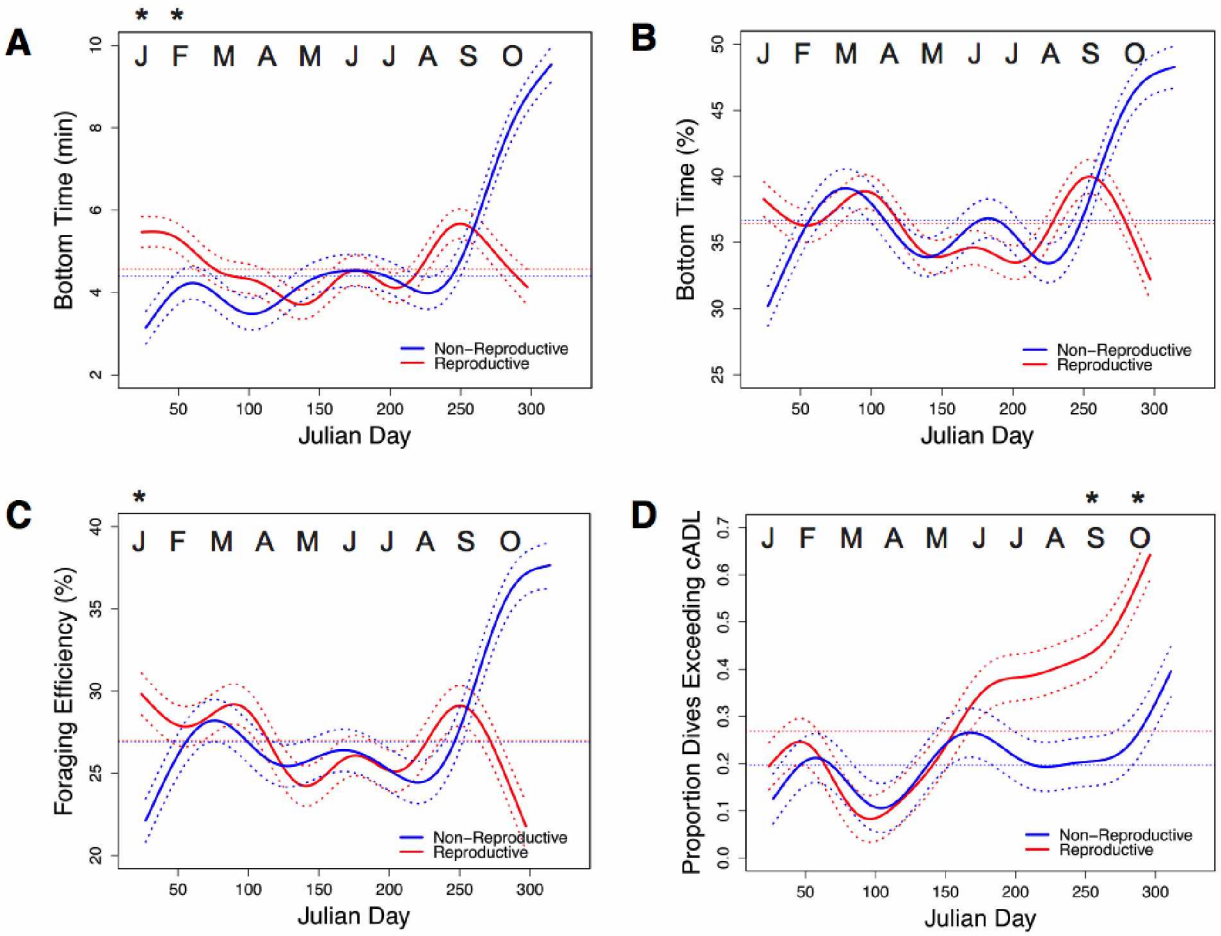


Figure 5.3. Generalized additive mixed models showing trends in bottom time (minutes and percent), foraging efficiency, and the proportion of dives exceeding the calculated aerobic dive limit (cADL) across the austral winter. Julian day had a significant effect on all dive parameters. Reproductive groups are shown as *blue* = Non-Reproductive; *red* = Reproductive. Month is abbreviated at the top of each panel and *Asterisk* = significant differences between reproductive groups for a given month. Horizontal lines represent means across the tag deployment.

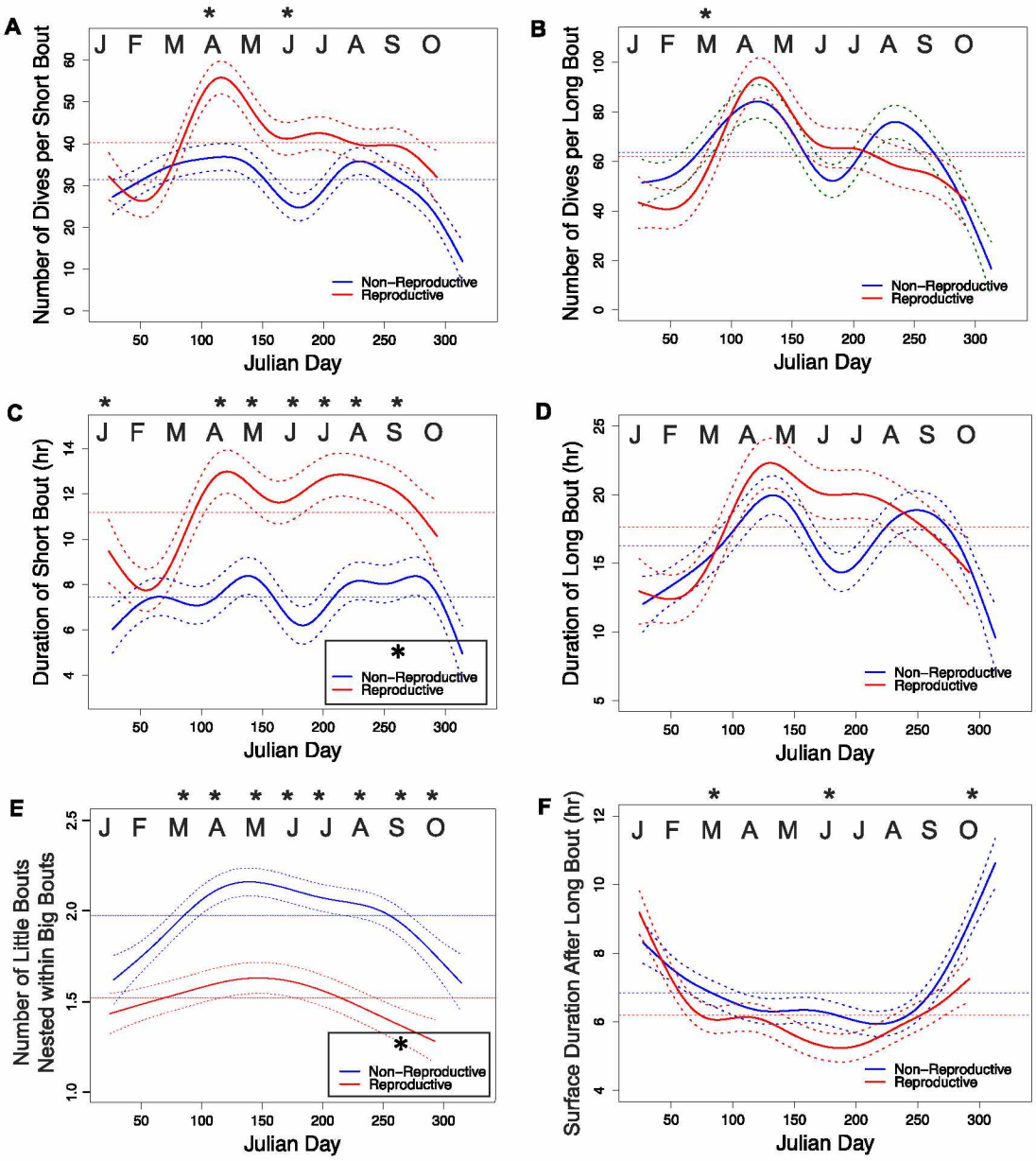


Figure 5.4. Generalized additive mixed models showing how the number of dives and bout duration changes across the year in $BOUT_{short}$ and $BOUT_{long}$ (**A-D**). The number of $BOUT_{short}$ nested within $BOUT_{long}$ differed across the year and by reproductive group (**E**) while surface duration exhibited an inverse relationship with bout durations (**F**). Reproductive groups are shown as *blue* = Non-Reproductive; *red* = Reproductive. Month is abbreviated at the top of each panel and *Asterisk* = significant differences between reproductive groups for a given month. *Boxed asterisk* = significant reproductive difference across the entire austral winter overall. Horizontal lines represent means across the tag deployment.

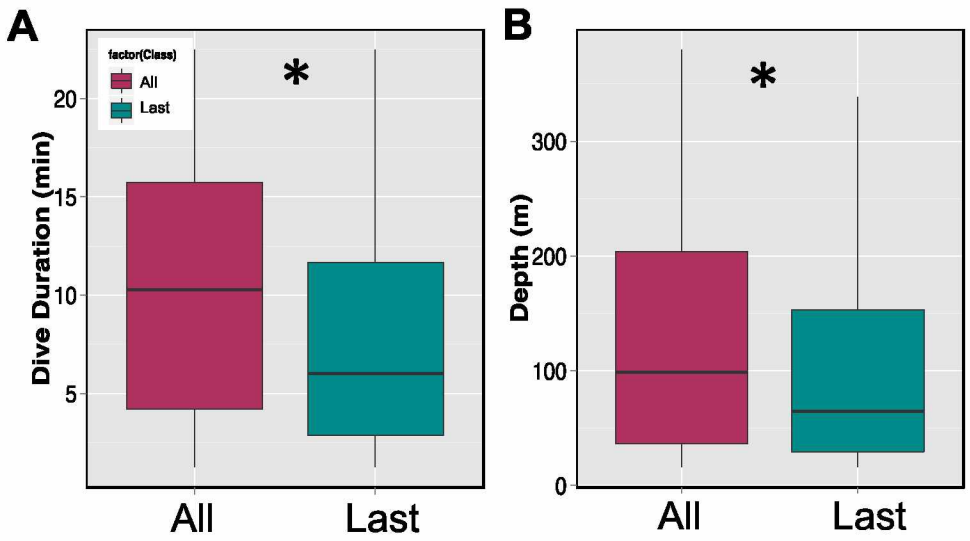


Figure 5.5. The duration and depth of the last dive in $BOUT_{short}$ were significantly shorter ($P < 0.001$) than the rest of dives in the bout.

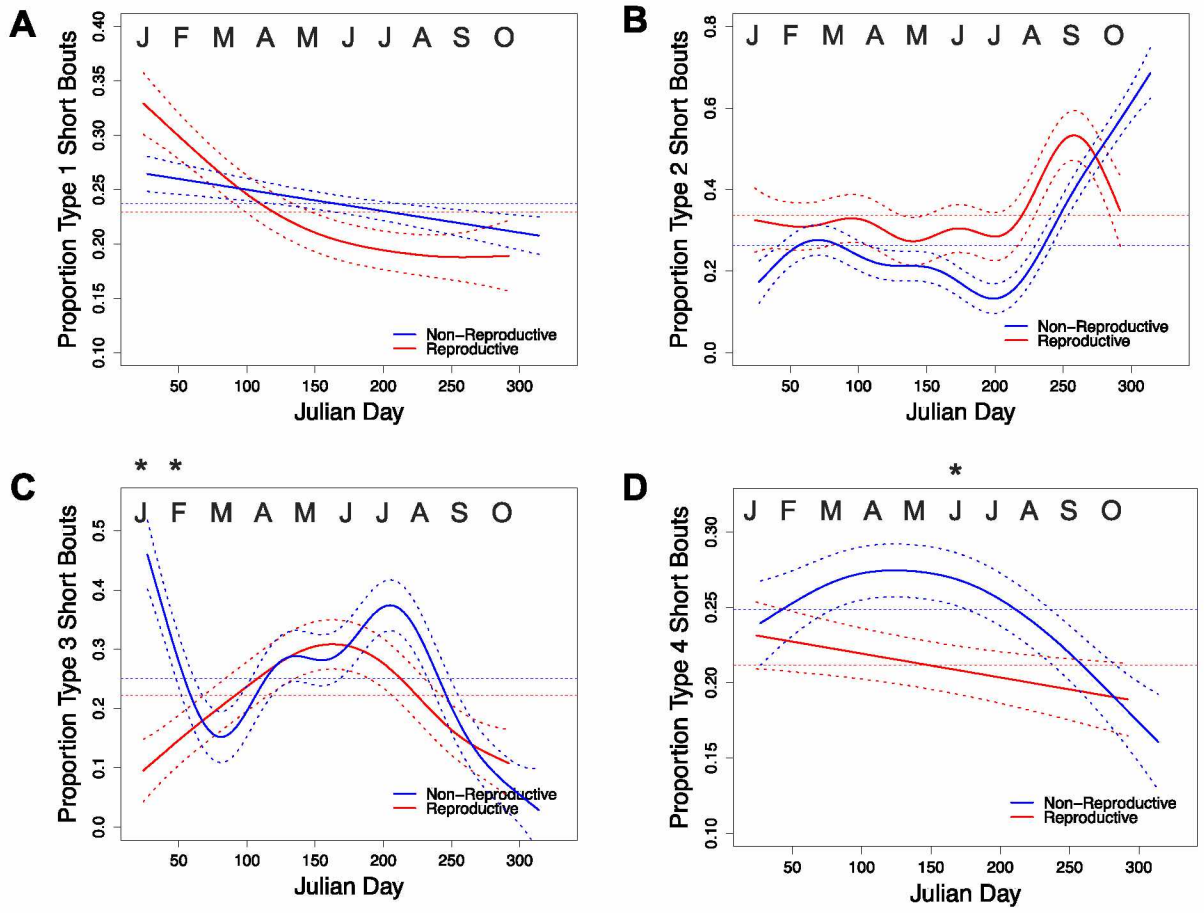


Figure 5.6. Generalized additive mixed models showing the proportion of $BOUT_{short}$ shapes across the year. All $BOUT_{short}$ types exhibit significant relationships with Julian day, but none differed by reproductive group. Reproductive groups are shown as *blue* = Non-Reproductive; *red* = Reproductive. Month is abbreviated at the top of each panel and *Asterisk* = significant differences between reproductive groups for a given month. Horizontal lines represent means across the tag deployment.

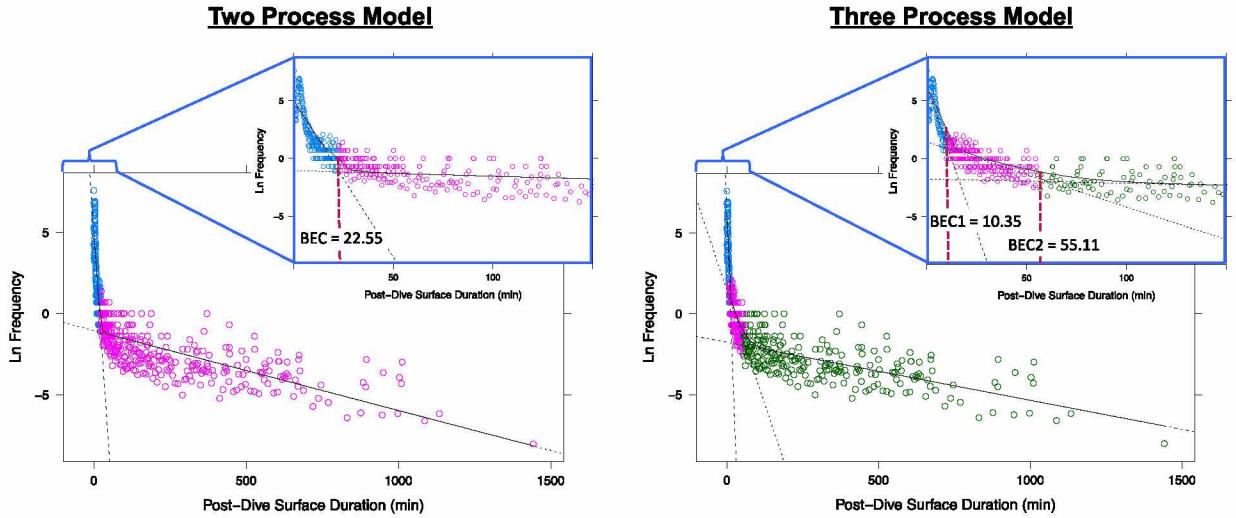
Table 5.1. Measured physiological parameters between fall post-molt and spring pre-breeding Weddell seals included in overwinter analyses of dive behavior.

	Fall (Post-Molt)					Spring (Pre-Breeding)				
Animal ID	**Mass (kg)**	**Lipid (%Mass)**	**TBO_2 (mL kg lean mass^{-1})**	**cADL (min)**	**LDH (IU g wet tissue^{-1})**	**Mass (kg)**	**Lipid (%Mass)**	**TBO_2 (mL kg lean mass^{-1})**	**cADL (min)**	**LDH (IU g wet tissue^{-1})**
Non-Reproductive										
WS10-03	214	27.5	111.9	17.2	580.3	---	---	---	---	762.1
WS10-05	271	28.4	128.9	20.8	630.5	---	---	---	---	---
WS10-07	234	32.3	149.3	21.9	288.9	303	37.0	118.8	17.3	370.1
WS11-08	277	34.5	109.8	16.3	561.2	---	---	---	---	519.1
WS12-04	440	31.8	103.2	17.9	474.5	475	34.4	127.4	21.6	739.2
WS12-08	429	38.1	103.0	16.1	509.9	---	---	---	---	---
WS12-13	410	34.2	109.7	18.0	482.5	408	38.5	117.6	18.0	477.8
WS12-19	381	29.5	118.7	20.5	791.5	437	33.1	114.0	19.3	747.2
Mean ± SE	331.9 ± 32.7	32.0 ± 1.25	116.8 ± 5.52	18.6 ± 0.78	539.9 ± 50.9	405.8 ± 36.9	35.8 ± 1.22	119.5 ± 2.84	19.1 ± 0.95	602.6 ± 68.7
Reproductive										
WS10-01	327	28.9	106.1	17.8	364.8	393	34.2	112.2	18.2	551.3
WS10-02	378	36.0	133.1	20.8	517.0	472	38.8	---	---	---
WS11-01	442	35.4	92.4	15.2	694.0	---	---	---	---	---
WS11-03	367	29.9	117.1	19.9	504.4	---	---	---	---	610.3
WS11-04	402	31.3	80.7	13.8	603.4	482	36.4	104.2	17.2	700.5
WS11-17	359	29.0	104.5	17.9	398.2	335	36.5	116.4	17.5	447.5
WS11-21	382	29.5	118.4	20.5	564.9	397	35.6	129.5	20.6	715.2
WS12-01	405	31.3	125.2	21.4	355.9	442	31.5	111.2	19.4	593.5
WS12-02	351	32.4	144.4	23.4	630.5	368	38.5	118.3	17.7	508.4
WS12-07	416	32.4	131.4	22.3	704.9	---	---	---	---	---
WS12-09	349	33.9	150.0	23.8	475.3	451	39.4	108.1	16.8	687.5
WS12-12	374	27.2	117.9	20.9	437.6	355	31.4	110.1	18.2	457.3
WS12-18	425	36.7	130.1	20.7	584.6	---	---	---	---	---
Mean ± SE	382.8 ± 9.28	31.8 ± 0.83	119.3 ± 5.47	19.9 ± 0.82	525.8 ± 32.5	410.6 ± 17.7	35.8 ± 0.99	113.8 ± 2.74	18.2 ± 0.44	585.7 ± 34.1
t-test (P value)	0.172	0.897	0.750	0.269	0.819	0.912	0.970	0.186	0.457	0.832

Table 5.2. Characteristics of Weddell seal dive shapes as determined by cluster analysis.

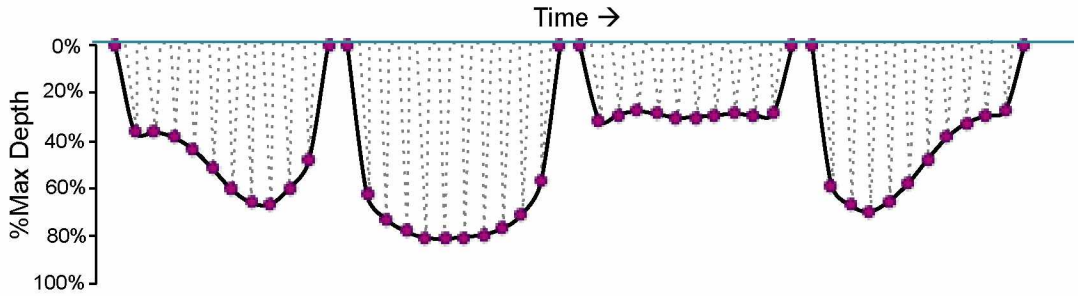
Dive Parameter	**Type 1** *Slow Descent/ Fast Ascent*	**2** *Fast Descent/ Slow Ascent*	**3** *Square-Shape*	**4** *V-Shape*
Frequency of Dive Type (%)	16.9 ± 0.8	16.1 ± 1.2	34.2 ± 2.0	32.7 ± 1.1
Dive Duration (min)	10.3 ± 0.52	12.3 ± 0.30	13.6 ± 0.50	10.1 ± 0.36
Maximum Depth (m)	83.1 ± 3.84	118.7 ± 5.18	154.7 ± 7.70	176.9 ± 9.26
Bottom Time (min; >80% Max Dive Depth)	1.96 ± 0.08	2.17 ± 0.07	7.26 ± 0.39	3.84 ± 0.17
Bottom Time (%Dive)	21.2 ± 0.35	18.6 ± 0.32	51.8 ± 0.95	36.5 ± 0.35
Ascent Rate (m/s)	0.78 ± 0.02	0.37 ± 0.01	0.93 ± 0.02	0.84 ± 0.03
Descent Rate (m/s)	0.45 ± 0.02	0.97 ± 0.05	1.13 ± 0.03	1.08 ± 0.04
Foraging Efficiency (%)	15.6 ± 0.26	13.9 ± 0.21	40.2 ± 0.94	25.3 ± 0.48

Table 5.3. Resulting bout ending criteria (BEC) values for two vs. three-process models. Akaike Information Criteria and ANOVA tests were used to assess whether adding a third process significantly improved model fit. In all Weddell seals, adding the third process improved models.



		Two Process Model		Three Process Model			Comparison of Models		
Animal ID	*Reproductive Status*	*BEC (min)*	*AICc*	*BEC1 (min)*	*BEC2 (min)*	*AICc*	*ΔAICc*	*ANOVA*	
WS10-01	Reproductive	22.55	1,682.7	10.35	55.11	1,526.8	-155.9	$F_{2,\ 153.7} = 91.4$	$P < 0.001$
WS10-02	Reproductive	26.87	2,181.5	17.53	50.23	2,111.0	-70.5	$F_{2,\ 62.9} = 38.8$	$P < 0.001$
WS10-03	Non-Reproductive	33.93	2,309.2	12.43	62.42	2,117.5	-191.7	$F_{2,\ 176.3} = 109.9$	$P < 0.001$
WS10-05	Non-Reproductive	46.76	2,429.3	13.85	77.28	2,255.4	-173.9	$F_{2,\ 136.7} = 97.7$	$P < 0.001$
WS10-07	Non-Reproductive	32.70	2,345.3	14.72	71.29	2,197.8	-147.5	$F_{2,\ 139.7} = 82.6$	$P < 0.001$
WS12-01	Reproductive	29.78	1,578.9	21.71	81.69	1,508.4	-70.5	$F_{2,\ 57.3} = 39.3$	$P < 0.001$
WS12-04	Non-Reproductive	38.97	2,338.1	21.62	111.68	2,147.0	-191.1	$F_{2,\ 161.4} = 109.0$	$P < 0.001$
WS12-09	Reproductive	30.89	2,251.9	16.58	80.74	2,147.9	-104.0	$F_{2,\ 108.6} = 57.6$	$P < 0.001$
WS12-12	Reproductive	29.80	1,890.8	16.50	81.48	1,751.7	-139.1	$F_{2,\ 127.0} = 79.1$	$P < 0.001$
WS12-13	Non-Reproductive	33.73	2,054.6	20.63	92.27	1,892.9	-161.7	$F_{2,\ 126.7} = 91.8$	$P < 0.001$

Table 5.4. Characteristics of Weddell seal $BOUT_{short}$ shapes as determined by cluster analysis, after interpolating maximum dive depths. $BOUT_{long}$ shapes were essentially identical, but consisted of a greater number of dives and were of longer duration (N = 10 for all parameters, except N = 7 for dives > cADL).



Bout Parameter	Type 1 *Left-skewed V*	2 *Deep, Square*	3 *Shallow, Square*	4 *Right-skewed V*
BOUT $_{short}$				
Frequency (%)	23.4 ± 0.94	30.0 ± 3.76	23.7 ± 3.12	22.9 ± 1.32
Number of Dives	36.0 ± 2.54	25.6 ± 1.91	45.2 ± 4.92	33.5 ± 2.25
Mean Dive Duration (min)	11.5 ± 0.6	14.7 ± 0.80	9.0 ± 0.39	10.9 ± 0.58
Total Dive Time in Bout (min)	432.8 ± 38.3	391.8 ± 35.8	427.6 ± 57.1	382.1 ± 34.5
Mean Surface Duration (min)	4.00 ± 0.21	4.41 ± 0.27	3.62 ± 0.19	3.90 ± 0.20
Total Surface Time in Bout (min)	124.0 ± 8.86	100.9 ± 8.36	144.1 ± 19.6	112.8 ± 8.96
Bout Dive : Surface Ratio	3.32 ± 0.20	3.86 ± 0.22	2.96 ± 0.24	3.27 ± 0.22
Bout Duration (hr)	9.42 ± 0.79	8.23 ± 0.72	9.69 ± 1.29	8.35 ± 0.71
Mean Depth (m)	141.9 ± 6.81	222.1 ± 14.7	93.4 ± 6.33	136.6 ± 6.53
Max Depth (m)	333.4 ± 18.3	334.8 ± 20.0	384.9 ± 27.6	340.8 ± 16.9
Dives Reaching >80% Bout Max Depth (%)	18.7 ± 0.67	48.7 ± 1.07	7.40 ± 0.38	18.7 ± 0.71
Post-Bout Surface (min)	195.0 ± 17.1	258.3 ± 33.2	229.1 ± 45.0	179.2 ± 10.2
Bout Efficiency (%)	14.0 ± 0.48	35.1 ± 1.18	5.37 ± 0.25	13.7 ± 0.59
Dives Exceeding cADL (%)	19.5 ± 3.20	30.8 ± 5.63	11.8 ± 2.06	17.6 ± 3.17

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Chapter 6. Physiological Condition and Dive Behavior Across the Gestation Period in Weddell Seals: Links with Reproductive Success of a High-Latitude Predator; Conclusions.

This dissertation provides novel longitudinal comparisons of the physiology and dive behavior across the gestation period in a polar marine mammal. Recent work has shown physiological plasticity across the year in marine mammals [1–4]. In particular, pinnipeds experience dramatic fluctuations in body mass and composition (i.e., lipid stores; [5–10]). In contrast, results from studies of the plasticity of endogenous oxygen storage capabilities appear inconsistent between the otariid and phocid families [1–3,11]. To my knowledge, this is the first marine mammal study to measure differences in physiological condition and dive efforts between individuals that give birth following the long foraging and gestation period, versus those animals that do not produce a pup.

Weddell seals showed resistance to the negative impacts of reduced exercise and maintained the calculated aerobic dive limit (cADL) throughout the year. Further, animals increased dive efforts and travelled 800 km to productive circumpolar deep water masses [12] during the austral winter. Still, despite conserving aerobic capacities and increasing dive efforts, Weddell seals only gained a fraction of the energy reserves that other phocid species acquire across gestation. There is evidence that prey capture success rates are lower in Weddell seals during the polar night [13], and therefore, animals may be increasing foraging efforts during the austral winter simply to gain equivalent amounts of prey, (i.e., they are "running to stay in place") as compared to the austral summer. Particularly for the Phocidae family that typically accrues most or all the necessary energy reserves to fuel the lactation period [14], gestating females may need to increase foraging efforts even further.

More recent work suggests that not all species in the Phocidae family adhere completely to a capital breeding strategy [8,15–18]. Northern elephant seals (*Mirounga angustirostris*), southern elephant seals (*Mirounga leonina*), and hooded seals (*Cystophora cristata*) completely fast throughout their lactation period [18,19]. Conversely, grey seals (*Halichoerus grypus*) can spend up to 8% [18], Weddell seals up to 25% [20], harp seals (*Pagophilus groenlandicus*) up to 47% [18], harbor seals (*Phoca vitulina*) up to 55% [21], ringed seals (*Pusa hispida*) up to 82% [18],

and bearded seals (*Erignathus barbatus*) up to 84% [18] of the day foraging while still nursing pups. Dive effort across lactation also appears to be graded, with females in poorer body condition diving more [22]. As with other phocid species, Weddell seals lose ~30% of their body mass during lactation, primarily as lipid mass [9]. This dissertation indicates that Weddell seals must forage during lactation, as they acquire less than half the required energy for nursing during the 8-month winter foraging period. Even more surprising was our finding that individuals that returned with a pup the following year did not store any greater total body or lipid mass towards "self" preparation than their non-reproductive counterparts. Even though gestating seals were preparing for an energetically costly lactation period, they had no more on-board energy reserves than skip-breeders; the only difference in calculated energetic gains across the austral winter was that associated with fetal growth [23]. Because Weddell seals drastically increase foraging efforts but still only gain modest energy reserves across the entire 8-month gestation period, this may make Weddell seals particularly vulnerable to prey and environmental disturbance during the winter months. This vulnerability may be reflected by the Weddell seal's relatively low reproductive output, as compared to other pinniped species.

Further, the finding that reproductive individuals did not start the gestation period in better condition also raises numerous questions regarding the mechanisms that initiate and maintain early pregnancy in this species. Because only fully-molted females were selected in January and February each year, none of the known-age animals [24–27] handled during the course of this study had given birth that year. That only the skip-breeders were fully-molted suggests that there is a physiological relationship between energetically-costly critical life history events such as lactation and molt (i.e., lactation delays the start of molt). This dissertation also suggests that hormones responsible for energy balance during the annual molt and the end of the embryonic diapause period are linked to reproductive outcomes. For example, animals that had higher thyroxine concentrations at the end of the molt were more likely to have a pup the next year, suggesting that this hormone may facilitate embryo attachment and maintenance of early gestation [28]. Successful reproductive outcomes the following year were also associated with higher growth hormone, likely preserving lean mass, and lower cortisol concentrations across the gestation period [23]. Future areas of study may benefit from determining the extent to which the Weddell seal's energetically costly life history events overlap temporally. For example, because this study suggests that the skip-breeders were molting earlier than post-partum females,

hormones associated with lactation (i.e., estrogen and prolactin [29–31]) or being in poor condition at the end of the lactation period may delay the initiation of the annual molt [32]. If the energetically-costly annual molt is also temporally separated from the start of gestation and the energetic costs associated with pregnancy, this temporal mismatch may ultimately contribute to the Weddell seal's high rate of skip-breeding relative to other pinnipeds [33]. Reproductive outcomes can also be influenced by female foraging success across the winter.

Adult female Weddell seals appear to have developed a suite of adaptations that make them resistant to the negative physiological impacts of reduced activity and rapid fluctuations in body composition. This work shows that adult female Weddell seals maintained all measured aerobic aspects of muscle biochemistry and structure (i.e., aerobic citrate synthase and β-hydroxyacyl-CoA dehydrogenase activities, and myosin heavy chain composition) necessary for efficient generation of propulsive power while foraging across the winter. Similarly, high concentrations of oxygen carrying proteins in the blood and muscle were also maintained. Either minimal exercise was sufficient to preserve elevated endogenous oxygen stores during periods of reduced activity throughout the austral summer, or this period was not long enough to lead to atrophy of oxygen stores. Whatever the mechanism, the ability to maintain aerobic capacity ensures that these animals can start off winter foraging at their fullest capabilities, when they are at their leanest. And indeed, this study found that dive duration, depth, and foraging efficiencies were higher just after the annual molt in January and February than early winter (March – April).

Mass was strongly correlated with many of the oxygen storage parameters. Somewhat unexpectedly, larger animals may need to expend less energy producing hemoglobin and red blood cells and instead have lower mass-specific total body oxygen stores as compared to smaller seals. Yet, this does not appear to put these larger animals at a disadvantage because their lower mass-specific diving metabolic rates (DMRs) resulted in equivalent calculated aerobic dive limits (cADLs) across the animal size range in this study [34–36]. Larger animals also dived longer during the first few weeks post-molt, and all animals increased the proportion of dives exceeding the cADL as winter progressed. During those months that the proportion of dives exceeding the cADL increased, depths that animals were reaching during dives also increased, indicating that animals may be targeting areas in the water column with greater abundance or caloric density of prey items. Mid-winter, reproductive females also significantly increased the

proportion of the day spent diving as compared to non-pregnant females. The percent increase in dive time closely mirrored the increase in energy acquisition I calculated that females would need in order to support the heat increment of gestation and accretion of fetal tissues [23]. Future study of seasonal changes in the Weddell seal's mass-specific diving metabolic rate (DMR), such as the potential to suppress metabolic rate during the annual molt [37] or during pregnancy [38] is needed to fully elucidate the changes in energetic expenditure across the year and by reproductive class.

Understanding the extent of physiological plasticity across the gestation period and the links between physiology and behavior is essential to understanding the ability of animals to extract sufficient energy from their environment to live and reproduce. In female Weddell seals, it appears that aerobic dive capacities are maintained across the year but that the relationship between physiology and dive behavior changes during the austral winter. Despite static aerobic capacities, animals dived significantly longer directly post-molt, mid-winter, and again just prior to the subsequent pupping season, suggesting that these particular time points may be critical to replenishing body mass and lipid reserves. This research has also made it clear that although animals maintain aerobic scope and start to function at (or surpassing) their calculated physiological limits, Weddell seals acquire less energy over winter than two other phocid species across their post-molt foraging period. Therefore, these animals must work and forage throughout the lactation period. Females must balance the need to transfer energy to the pup while also minimizing detrimental effects to the female and maintaining a threshold body condition. This dissertation also makes it clear that a great deal of work still needs to be done in assessing what intrinsic mechanisms regulate (1) the start of active gestation, (2) the maintenance of pregnancy to full-term, and (3) how animals are able to greatly exceed physiological dive limits to attain the energy necessary to fulfill life history events. Being able to quantify increases in foraging efforts and energy that must be extracted from the environment to support reproduction in a top marine predator becomes increasingly important as climate change progresses, and with the establishment of major fisheries within the Ross Sea [39,40].

6.1 References

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Appendix A.

Co-Author Approvals to Include Published Manuscripts in This Dissertation

Permission of Co-Authored Manuscript Inclusion in UAF Dissertation

As co-author on the manuscript "Improving the Precision of Our Ecosystem Calipers: A Modified Morphometric Technique for Estimating Marine Mammal Mass and Body Composition" that has been published in PLoS One, I give Michelle Shero permission to include this paper as a chapter in her graduate student dissertation to the University of Alaska Fairbanks.

Citation: Shero, M.R., L.E. Pearson, D.P. Costa, and J.M. Burns. 2014. Improving the precision of our ecosystem calipers: A modified morphometric technique for estimating marine mammal mass and body composition. PLoS One 9(3): e91233. DOI: 10.1371/journal.pone.0091233.

Co-author: Linnea E. Pearson

Signature: Linnea Pearson Digitally signed by Linnea Pearson DN: cn=Linnea Pearson, o=University of Alaska Fairbanks, ou, email=lepearson@alaska.edu, c=US Date: 2015.06.10 21:38:14 -08'00' Date: 6/10/15

Permission of Co-Authored Manuscript Inclusion in UAF Dissertation

As co-author on the manuscript "How do overwinter changes in body condition and hormone profiles influence Weddell seal reproductive success?" that has been published in Functional Ecology, I give Michelle Shero permission to include this paper as a chapter in her graduate student dissertation to the University of Alaska Fairbanks.

Citation: Shero, M.R., R.T. Krotz, D.P. Costa, J.P. Avery, and J.M. Burns. 2015. How do overwinter changes in body condition and hormone profiles influence Weddell seal reproductive success?. Functional Ecology DOI: 10.1111/1365-2435.12434.

Co-author: Julie P. Avery

Signature: _____ Date: 6.8.15

Co-author: Riley T. Krotz

Signature: _____ Date: 6-8-15

Permission of Co-Authored Manuscript Inclusion in UAF Dissertation

As co-author on the manuscript "Temporal changes in Weddell seal dive behavior over winter: Are females increasing foraging efforts to support gestation?" that is currently being prepared for publication, I give Michelle Shero permission to include this paper as a chapter in her graduate student dissertation to the University of Alaska Fairbanks.

Citation: Shero, M.R., K.T. Goetz, D.P. Costa, and J.M. Burns. 2015. Temporal changes in Weddell seal dive behavior over winter: Are females increasing foraging efforts to support gestation?. Prepared for submission to Marine Ecology Progress Series.

Co-author: Kimberly T. Goetz

Signature: _____ Date: June 18, 2015

Appendix B.

IACUC and NMFS Marine Mammal Permit Approvals

Use of Live Vertebrate Animal Subjects with Contact 1

Institutional Animal Care & Use Committee (IACUC) **CODE: Costd0906**

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- Refer to instructions for most items at: http://carc.ucsc.edu/process.html
- Tailor your responses to the questions; do not change questions.
- Be consistent with any grant application, but do not reference or attach it.
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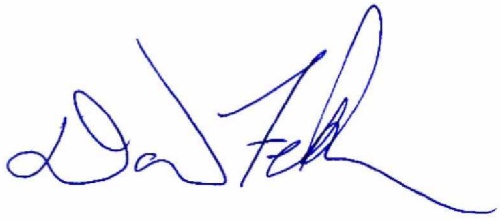
Download the latest IACUC application form at: http://carc.ucsc.edu/download.html. Applications prepared on old forms will not be accepted by the IACUC for review. All personnel named on this protocol application and subsequent amendments must have completed the IACUC training requirement showing an understanding of federal laws and policies. For more information, contact the IACUC Administrator (carc@ucsc.edu, 9-3150) or go to: http://carc.ucsc.edu/WebTraining.html						
1. Activity title: Collaborative Research: Weddell Seals as Autonomous Sensors of the Winter Oceanography of the Ross Sea						
2. Principal investigator/project director name: **Daniel P Costa**					Title: **Professor**	
Department or unit: **Ecology Evolutionary Bio**			Email: **costa@biology**		Phone: **9-2786**	
Co-Respondent (as alternate for PI): **Kim Goetz**			Email: **goetz@biology**		Phone: **9-3112**	
MailStop: **LML_COH**						
3. Purpose:	**Research**	**Grant**				
If this is a class, specify course name and number:						
4. Today's date: **6/29/09**	Estimated animal use start date: **1/15/10**			Estimated animal use end date:**2/28/13**		
5. Current Funding agency: **NSF OPP**			Grant start date: **7/1/09**		Grant end date:**6/30/13**	
IF you have applied through OSP (Office of Sponsored Projects) for funding for a grant covered by this application, specify your grant title and the OSP data sheet SC number corresponding to that title. Your OSP analyst can give you the SC number. **Failure to provide correct information may delay distribution:**						
SC#:20081155	Title: Collaborative Research: Weddell Seals as Autonomous Sensors of the Winter Oceanography of the Ross Sea					
Is IACUC verification letter required? **YES /** If yes, contact IACUC Administrator 2 weeks in advance.						
6. Does this document contain proprietary material? **NO**						

I, the undersigned principal investigator or project director, am familiar with the PHS Policy on Animal Care and Use and the NIH Guide. I accept full responsibility that all animal use in this activity will conform to these and all other relevant regulations. I assure that the information in this application is fully and accurately presented and conforms to my representations to any sponsoring agency; that any pain and distress to animals will be limited to that which is unavoidable in the conduct of scientifically valuable research; that analgesic, anesthetic, and tranquilizing drugs will be used where indicated and appropriate to minimize discomfort and pain to animals; that no activities in this application unnecessarily duplicate previous experiments. I understand that this activity is not approved until all conditions are met and I receive written notification, and that any proposed changes in site, personnel, objective, procedures, species, or animal numbers must be approved by IACUC before implementation.

See PI email **7/1/09**

Signature of Principal Investigator *Date*



Signature of chair or designated reviewer signifies approval of majority of the IACUC *Date*

Research & Graduate Studies
University of Alaska Anchorage

3211 Providence Drive
Anchorage, Alaska 99508-4614
T 907.786.1099, F 907.786.1791
www.uaa.alaska.edu/research/ric

DATE:	June 21, 2010
TO:	Jennifer Burns, PhD
FROM:	University of Alaska Anchorage IACUC
STUDY TITLE:	Weddell seals at Autonomous sensors of the winter oceanography of the Ross Sea
IRB REFERENCE #:	177250-1
SUBMISSION TYPE:	Addition of New Personnel
ACTION:	APPROVED
APPROVAL DATE:	June 21, 2010
EXPIRATION DATE:	July 2, 2012
REVIEW TYPE:	Administrative Review

Your Request for Addition of New Personnel (addendum to Protocol# 177250; Original UAA IACUC# 2009Burns1) was approved via Administrative Review. **Michelle Shero** is, therefore, authorized to conduct research on vertebrate animals according to your existing protocol.

We remind you that all changes in personnel and animal handling protocols must be submitted to the committee prior to these changes taking place. In addition, should you experience any unexpected animal mortalities, illnesses, or injury (to animals or personnel involved with the project), you are required to report such to the IACUC immediately. Guidelines for this notification are attached.

Thank you for your support of animal care guidelines.

Eric S. Murphy

Eric S. Murphy, Ph.D.

Chair, UAA IACUC

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University of Alaska Anchorage

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Anchorage, Alaska 99508-4614
T 907.786.1099, F 907.786.1791
www.uaa.alaska.edu/research/ric

DATE:	May 15, 2013
TO:	Jennifer Burns, PhD
FROM:	University of Alaska Anchorage IACUC
PROJECT TITLE:	[346067-3] Collaborative Research: Weddell Seals as Autonomous Sensors of the Winter Oceanography of the Ross Sea
IRBNET REFERENCE #:	346067-3
SUBMISSION TYPE:	Closure/Final Report
ACTION:	APPROVED
APPROVAL DATE:	May 10, 2013
EXPIRATION DATE:	
REVIEW TYPE:	Full Committee Review

The final report to the Institutional Care and Animal Use Committee regarding your study has been approved. Thank you for your support of animal care guidelines and congratulations on the conclusion of your research.

Tim Hinterberger, Ph.D.
Assoc. Professor, WWAMI School of Medical Education
Chair, Institutional Animal Care & Use Committee, University of Alaska Anchorage
3211 Providence Drive
Anchorage, Alaska 99508

UNITED STATES DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
NATIONAL MARINE FISHERIES SERVICE
Silver Spring, MD 20910

NOV 0 4 2011

Daniel P. Costa, Ph.D.
Long Marine Laboratory
University of California at Santa Cruz
100 Shaffer Road
Santa Cruz, California 95060

Dear Dr. Costa:

The National Marine Fisheries Service has issued Permit No. 87-1851-04, which amends and replaces Permit No. 87-1851-03, for research activities on marine mammals. The changes to specific Terms and Conditions are reflected in bold font. This permit is effective upon your signature and valid through the expiration date of December 31, 2012.

You must return the "file copy" signature page, with your dated signature, to this office as proof of your acceptance of the permit. There are two signature pages in the enclosed permit. Please sign and date both pages. Keep the original signature page with the rest of the permit as proof of your authorization to conduct the research activities.

Please read your permit carefully before signing it. If you have questions, please contact your permit analyst – Amy Sloan or Tammy Adams – at 301-427-8401 before signing the permit. If you need assistance with your permit in the future, please contact one of these permit analysts.

Please return the signature page marked "file copy" to the Chief, Permits Division (F/PR1), 1315 East-West Highway, Silver Spring, MD 20910. You may also submit the "file copy" of the signature page by email (Amy.Sloan@noaa.gov) or fax (301-713-0376) and confirm it by mail.

Sincerely,

P. Michael Payne
Chief, Permits, Conservation
and Education Division
Office of Protected Resources
(phone: 301-427-8401)

Enclosure